The Journal of Laboratory and Clinical Medicine

VOL. 28 NOVEMBER, 1942 NO. 2

PROGRESS

ANTIBODY PRODUCTION AND THE ANAMNESTIC REACTION*

PAUL R. CANNON, M.D., CHICAGO, ILL.

SOME important aspects of antibody production have remained obscure for many years because of the doubt concerning the chemical nature of antibodies. The problem of antibody action, therefore, has received more attention than has that of antibody structure. Within the past few years, however, new facts about the chemical constitution of antibodies have suggested new methods of approach to the general problem of antibody formation. The resultant rapidly changing viewpoint can be briefly summarized as follows: In 1929 Wells,[1] in expressing the commonly held opinion of that time, said, "We have absolutely no knowledge what these antibodies may be, or even that they exist as material objects. Like the enzymes, we recognize them by what they do without knowing just what they are. We do not know whether they are specific molecular aggregates or merely physical forces dependent on altered surface energy of the same substances which were already present in the blood before the process of immunization was even begun." Zinsser, two years later, indicated a changing point of view:[2] "It is generally assumed that antibodies cannot be separated from proteins and are usually contained in the pseudoglobulin fraction, in some cases in the euglobulin fractions of the sera." By 1938 Heidelberger had stated the current view in these words:[3] "Although writers on immunology are still to be found who prefer to consider antibodies as ideas, rather than chemical substances, there is now much evidence that antibodies actually are modified serum globulins." And Marrack has recently said (1942):[4] "Now, however, that a large volume of evidence has accumulated which shows that antibodies are modified serum globulins, the chief problem has become the structure and properties of proteins in general and the special characteristics of antibodies in particular." With the chemical composition of antibodies thus definitely established

*From the Department of Pathology, the University of Chicago.

Some of the work on which this discussion is based was aided by the Douglas Smith Foundation for Medical Research of the University of Chicago and the John and Mary R. Markle Foundation.

it should now be possible to proceed more confidently to the study of the conditions that influence their production.

Present-day theories regarding the formation of antibodies all agree in postulating their intracellular production from globulin. The molecules of globulin are synthesized in stereochemical relationship to antigen, and when released, exhibit specific affinities for this antigen. The theory of Breinl and Haurowitz[5] assumes that during the process of antibody formation antigens enter into the mixture of amino acids and peptides and induce new groupings. The formation of antibody thus represents the synthesis of a special kind of globulin in which the molecules orient themselves in ways characteristic for each antigen. As the antibodies pass into the blood the antigen remains behind, thereby continuing to influence the formation of new antibody globulin until the antigenic components are destroyed or eliminated from the antibody-producing cells. Alexander[6] postulates the development within cells of "new specific catalysts" capable of directing antibody synthesis. During their development the antigen becomes an "essential part of the directive surface of the catalyst particles, thereby leading to the formation of groups of particles with reversed electrostatic charge patterns." By such a mechanism minute quantities of antigen, even as little as one molecule, can "convert a cell or an extracellular catalyst into a potential producer of antibody." Mudd's theory[7] embodies the idea that antibodies are synthesized from peptides and amino acids in an "orienting environment, namely, the antigen-protoplasm interface." Amino acids which become coupled to the growing antibody molecule must conform to the "spatial and chemical requirements of the interface in which synthesis is proceeding." Therefore, the antibody corresponds structurally to the configurations of the antigenic surface and hence is specific, i.e., after dissociation it can recombine with antigenic molecules of similar structure. Furthermore, after dissociation the antigen again is available to act as a directive pattern for further synthesis of antibody. Pauling[8] has suggested recently that during antibody formation the molecules of antigen act as directive patterns or templates in the process of globulin synthesis; consequently, a molecule of antibody differs from a molecule of normal globulin only in the portion of the coiled polypeptide chain which has been "templated" during synthesis by contact with portions of the antigen. According to this concept normal globulin and antibody globulin are essentially identical chemically but differ structurally somewhat as one automobile tire may differ from another in tread pattern although being chemically similar. Further verification of this concept is suggested by the recent experiments of Pauling and Campbell,[9, 10] demonstrating the formation of specific antibodies in the test tube by a process of "unfolding" of polypeptide chains, followed by a "refolding" in the presence of antigen. Later dissociation of the complex releases the antibody globulin molecules which can then recombine with their specific antigen.

Inasmuch as these theories and the facts on which they are based all warrant the conclusion that antibody globulin is specifically modified normal globulin, it becomes evident that the mechanism of antibody formation is closely related to that of globulin production. Furthermore, if both types of globulin arise from the same source, deficiencies in the formation of the one must be related to deficiencies in the production of the other. It would seem, therefore, that a clearer understanding of the conditions determining the production of

globulins should help to elucidate both the nature of the antibody mechanism and the processes of antibody production.

Much progress has been made in recent years in the search for the origin of the serum proteins, and many workers have emphasized the importance of the liver; but, as has been pointed out,[11] contradictory experimental results make it impossible to conclude that the liver is the sole source. Reimann, Medes, and Fisher, in their review of the subject,[11] remarked: "It would, however, seem reasonable to search for the site of origin of blood proteins in the blood-forming organs and, for this reason, the bone marrow, the endothelial system including the liver and spleen, and the cellular elements of the blood have been widely studied. Since it is now generally accepted that bilirubin arises from hemoglobin after the dissolution of erythrocytes, it seems plausible to suggest that the plasma proteins might arise in a similar manner from certain cells in the circulation or in the bone marrow, or from tissue cells generally. It would be in the nature of biologic economy to utilize the substances thus liberated, and certain experimental and presumptive evidence supports this view."

Evidence substantiating this suggestion of Reimann, Medes, and Fisher has come both from observation and experiment. Thus Downey and Weidenreich[12] observed the shedding and dissolution of protoplasmic material from lymphocytes and plasma cells and thought that this might indicate a secretory activity of these cells. Sabin[13] reasoned from her earlier studies on the origin of the blood vessels in the chick blastoderm that "since for a vessel to function as such it is essential for the plasma to contain some protein to maintain the balance of intra- and extracellular fluid, the original transformation of a solid mass of angioblasts into a vessel involves the sacrifice of some cytoplasm to make the serum proteins." Her later experiments utilized "marked antigen" of alum-precipitated dye protein, the fate of which, when it was injected into rabbits, could be seen within macrophages. She observed that "coincident with the time when the dye protein is no longer visible within the cells and when there are antibodies in the serum, there is a marked shedding of the surface films of the macrophages without damage to them." If this process of cytoplasmic shedding is a general property of globulin-producing cells, any such cell which can phagocytose antigen or allow its entrance into the cytoplasm may be a precursor of antibody; furthermore, a part of the antibody globulin may remain within the cell for long periods of time instead of being "sacrificed" as blood protein to the surrounding fluids. Landsteiner and Parker[14] have shown, in tissue culture, that in the fluids resulting from the growth of connective tissue cells of chicks in rabbit plasma, proteins are formed which react by precipitin tests with sera from rabbits injected with chicken serum. No attempts were made to determine whether the proteins were albumins or globulins, but the experiments furnished presumptive evidence that connective tissue cells can form serum proteins. It would be of particular interest to know to what extent such cells can produce globulins; their inability to do so adequately and continuously under the artificial conditions of tissue culture might explain the numerous conflicting results of attempts to produce antibodies in vitro.

The idea of the intracellular origin of globulin becomes especially important in connection with the possibility that immune reactions may occur independently of the presence of antibodies in the blood. Besredka,[15] in his formu-

lation of the concept of local immunity, postulated a local tissue immunity without the obligatory participation of antibodies in the circulating fluids. Kahn[16] suggested that specifically immunized tissues may acquire a capacity for union with antigen independent of the presence of antibodies in the blood serum. Seegal and Seegal[17] demonstrated antibodies in ocular tissues in the absence of demonstrable antibodies in the adjacent extracellular fluids. Dienes and Mallory[18] pointed out that hypersensitiveness of the tuberculin type may occur independently of the presence of humoral antibodies and concluded that "if circulating antibodies are involved in the mechanism of this type of hypersensitivity they must be of an unknown nature or else exist in the blood stream in some as yet undetectable form. It appears more probable that we are dealing with an altered tissue reactivity." Walsh and Cannon[19, 20] concluded that regional immunization may lead to the formation or accumulation of antibodies in locally immunized tissues in concentrations higher than in other organs of the same animal and at times even higher than in the blood serum. Although the above workers have all used methods which are admittedly inadequate in some respects, sufficient evidence exists, nevertheless, to indicate the presence of changed reaction capacities of tissues which do not parallel the antibody content of the blood. Inasmuch as the solution of many of the problems of allergy and of tissue immunity depends upon the understanding of the relative influences of cellular and humoral immune reactions, continued efforts must be made to determine their respective potentialities.

An immune phenomenon hitherto not adequately studied is the anamnestic reaction. This phenomenon, while generally recognized as a source of error in the interpretation of certain laboratory procedures, has not received the theoretical attention it deserves. The name "anamnestische serum reaktion" resulted from observations by Conradi and Bieling[21] in 1916 that soldiers previously vaccinated with the typhoid vaccine had a sharp rise in titers in the Gruber-Widal reactions in the course of febrile diseases that stimulated typhoid fever, but that later proved to be something else, as for example, typhus, miliary tuberculosis, pneumonia, erysipelas, bacillary dysentery, Weil's disease, etc. Conradi and Bieling demonstrated in rabbits immunized against typhoid bacilli that the later injection of colon, diphtheria, or dysentery bacilli also caused a sharp rise in the titer of the serum against typhoid bacilli. They concluded, therefore, that the stimulation of antibody-producing cells by heterologous microorganisms caused them to "recall" their previous production of antibodies to typhoid bacilli.

These observations of Conradi and Bieling, although not new, served to emphasize the significance of earlier ones. Dieudonné,[22] for example, as early as 1906 had shown that the injection of sodium cinnamic acid into typhoid-immunized rabbits with low agglutinative titers caused a renewed formation of agglutinins. This demonstration apparently aroused but little interest until the diagnostic problems arising in World War I called renewed attention to the importance of the anamnestic reaction. Thus, Fleckseder[23] in 1916 concluded that many kinds of febrile diseases could cause a sharp rise in the concentration of agglutinins to the typhoid bacillus in the serum of immunized persons, and Weil and Felix[24] saw a rise in titers in the Gruber-Widal reaction in approximately 53 per cent of patients in the course of typhus, as well as to some degree

in other infectious processes. In 1917 Dreyer and Inman[25] emphasized the importance of quantitative methods in agglutination tests in typhoid-immunized persons with enteric disease because of the concomitant production of major and minor agglutinins. In the same year Baehr[26] said: "It has been occasionally noted that the Widal reaction may become positive during any febrile disorder in persons who previously had typhoid fever or who have recently been vaccinated with typhoid bacilli. . . This temporary lighting up of the Widal reaction during typhus fever was commonly observed by the German and Austro-Hungarian army bacteriologists in Russian Poland and Volhynia, for all the troops had received typhoid vaccinations." It was not known until many years later that the capacity to produce agglutinins to the typhoid bacillus after immunization may persist in healthy persons for as long as from six to nine years.[27]

The demonstration by Dieudonné that nonspecific agents can elicit the anamnestic response was confirmed in 1917 by Kirstein[28] in his experiments showing that infection of typhoid-immunized rabbits with vaccinia virus also caused a mobilization of agglutinins to the typhoid bacillus. He emphasized particularly the fact that nonbacterial agents had a stimulative effect. In all of these earlier observations, however, the primary emphasis was on the practical aspects of the reaction because of the diagnostic difficulties presented, and only a few efforts were made to ascertain the cause of the anamnesis. Some attributed the reaction to the reappearance of "preformed antibodies";[26] others thought that the acute hyperplasia of the spleen was responsible; only Conradi and Bieling made any attempt to determine experimentally the underlying nature of the phenomenon.

The anamnestic phenomenon can be demonstrated, also, against nonbacterial antigens. Obermeyer and Pick[29] in 1904 observed the reappearance of precipitins to beef serum after injection of horse albumoses in rabbits previously injected with beef serum. Little attention was paid to this aspect, however, until 1917, when Hektoen[30] pointed out an important source of error in the production of precipitins for foreign sera in that, when rabbits were injected with one type of serum and were later injected with a heterologous serum, precipitins to the first serum reappeared. He said at that time: "The particular point on which special stress is placed now is the capacity of the rabbit under suitable conditions . . . to elaborate different precipitins at the same time. It appears that the precipitin production induced in the usual way leaves behind it an increased power of further production so that large amounts of major as well as group and minor precipitins are elaborated on the injection of a new serum or blood. Manifestly, the phenomenon is an expression of an increased reactivity and may be classed with other manifestations of allergy." Bieling,[31] in later experiments, re-emphasized the idea that the changed reaction capacity of tissues is an expression of allergy whereby the power of a cell to produce antibody can be changed both specifically and nonspecifically. He visualized the immune state in a cell as a process of development of a nonspecific hypersusceptibility to other kinds of stimuli; as a result of this, later stimuli can engender the appearance of nonspecific antibodies in the pretreated animal. Mackenzie and Frühbauer[32] reported that after precipitins to foreign proteins had disappeared from the blood, later injection of another protein caused them to

reappear; furthermore, the precipitins to the last antigen previously injected were the first to reappear. Hektoen and Welker[33] have shown, also, that subsequent to the induction and disappearance of multiple precipitin formation by the successive introduction of different antigens, the injection of only one of the antigens previously used may cause the reappearance of precipitins to the same as well as the other antigens.

During the entire period of study of the anamnestic reaction, unnecessary confusion has centered around the use of the term itself. The word "anamnesis" means "a recalling," and in the anamnestic reaction the antibody-producing cells presumably "recall" or "remember" a previous antigenic experience during the course of a subsequent and frequently unrelated type of cellular stimulation. Because of the heterologous nature of the later stimulus too sharp a restriction has been placed upon this feature of the phenomenon. As Topley[34] has said: "In this connection we note the terminological confusion which has sometimes arisen in the literature. The production, in response to an antigenic stimulus, of an antibody that has been produced in the tissues on some previous occasion is often referred to as an anamnestic reaction (anamnesis recollection). Sometimes this term is used in connection with the type of nonspecific stimulus to which we have just referred—the tissues are stimulated to general activity, in which is included the renewed production of a particular antibody that has been formed at some time in the past. Sometimes, perhaps more accurately, it is employed in reference only to those instances in which the particular chemical stimulus concerned formed part of the earlier experience of the antibody-producing cells." There can be no doubt, therefore, that Topley applies the term "anamnestic reaction" both to the nonspecific and the specific liberation of antibodies into the blood stream consequent to a later antigenic stimulation. This broader point of view implies, therefore, that if antibody-producing cells are to manifest some type of "recollection" of an earlier antigenic experience, there is no reason why this should happen only after a nonspecific stimulation. The determining feature of the anamnestic reaction is the speedy release of antibodies from their site of production, regardless of the stimulus which may bring it about. Acceptance of this point of view makes it immaterial whether the reaction is called "anamnestic," "secondary immune response," "injection de rappel," or the reaction to "stimulating," "booster," or "repeat" injections.

In the present discussion the anamnestic reaction is considered to be the result either of nonspecific or specific stimulation of antibody-producing cells which have undergone earlier antigenic stimulation. After nonspecific stimulation there is the reappearance of antibodies to the earlier antigen; after specific stimulation there is an earlier and more abundant reappearance of antibodies to the specific antigenic stimulant.

The importance of the anamnestic phenomenon is seen also in infectious processes. For example, the use which is now being made of the so-called "stimulating" or "booster" injections of toxoids in order to call forth an accelerated liberation of antitoxins[35-38] is an instance of "specific anamnesis," whereby higher levels of antitoxic immunity are attained in children and in soldiers who have been immunized previously with toxoids of diphtheria and tetanus. Jones and Moss describe the case of a person who eight months after immunization with tetanus toxoid suffered a puncture wound of the hand.

His blood serum, when tested after immunization, had shown the following antitoxic levels: at six weeks, one-tenth unit per cubic centimeter; at twelve weeks, one-twentieth unit; at six months, one-fortieth unit. On the day following the injury he was reinjected with 0.25 c.c. of alum-precipitated tetanus toxoid; his blood serum, tested one week later, contained one-tenth unit per cubic centimeter, and four weeks later, one-half unit. The authors estimated that his total blood, therefore, would have contained approximately 3,000 units of antitoxin, or "more than enough antitoxin to protect against the development of clinical tetanus" as well as to possess an active immunity far superior to the passive protection which would have been possible by the injection of antitoxin. These authors showed later, moreover, that stimulating injections of toxoid not only accomplished the accelerated elevation of antitoxic levels in the blood, but that these levels persisted longer than after first injections. It is not unlikely that in persons adequately immunized with toxoids a later infection with diphtheria or tetanus bacilli will, because of the speedy liberation of small quantities of toxin into the tissues, engender an accelerated secondary response and thus bring freshly liberated antitoxins quickly to the site of developing infection, thereby favoring an early termination of the infective focus.

The anamnestic reaction may operate similarly in infections in which antigenic stimulations accompanying the acute infectious process may liberate, in varying degrees, antibodies engendered by earlier antigenic experiences. It is conceivable that such a response would help materially in restraining the development of incipient secondary infections which might otherwise complicate seriously the major infectious process. For example, Hektoen and Boor[39] called attention to the fact that "the serum of apparently healthy persons contains, as is well known, small quantities of a great variety of antibodies for infectious, as well as non-infectious, antigens . . . it seems reasonable to assume that 'normal antibodies' may develop in response to antigens that enter the blood from the digestive and respiratory tracts, as well as in other ways."

It may be recalled that during the first World War the most serious complications of influenza and measles occurred in soldiers from the sparsely settled rural areas. These men, presumably, had had fewer antigenic experiences with microorganisms commonly implicated in respiratory infections than had soldiers coming from the thickly populated urban areas. It is possible that, during the course of influenza or measles, the anamnestic mobilization of multiple antibodies to various types of streptococci and pneumococci may have played an important part in restricting the early growth and spread of secondary bacterial invaders which might otherwise have caused serious or even lethal infections.

The anamnestic reaction may also play a beneficial role in the treatment of chronic or focal infections in which, presumably, foci of infective agents persist in the tissues over long periods of time. For example, in the treatment of chronic syphilis, arthritis, or iritis, stimulation of the antibody mechanism by various foreign protein materials, such as typhoid vaccine, milk, malarial parasites, or by hyperthermic means, may cause the liberation into the blood stream of significant amounts of specific antibodies engendered by previous antigenic experiences but which could not otherwise gain access to the infected foci. According to Cecil,[40] "perhaps the most important function of the foreign protein reaction is the mobilization of immune bodies in the circulating blood. . . . Indeed, it has

been shown by numerous authors that any form of fever is conducive to an increase of immune bodies in the circulating blood. Whether this is due to an increased formation or to increased mobilization of antibodies it is difficult to say." O'Leary[41] also remarks: "The literature contains numerous hypotheses as to the mechanism that produces the satisfactory results of fever therapy, but not one of these offers sufficient evidence to warrant its acceptance except as a theory. In the fever induced by the malarial treatment, as well as in that produced by other nonspecific agents, it is my impression that the satisfactory therapeutic effects are the result of some fundamental change in the immunologic processes, the nature of which is unfamiliar. The high temperatures which are produced may be a factor in bringing about these changes." Although more examples might be cited, these will suffice to illustrate the general importance of the phenomenon, not only through its effects upon various laboratory procedures, but also upon many important problems of acquired resistance.

Perhaps, however, the relationship of the anamnestic reaction to the problem of antibody production is of even greater significance. Although it is now generally agreed that the antibody-producing mechanism consists predominantly of macrophages comprising the reticulo-endothelial system, it is possible that antibody formation may be more widespread. In fact, any cell which can actively phagocytose foreign antigenic materials, or even allow them to enter its cytoplasm and remain there while globulin is being synthesized may, presumably, either produce antibody for release into the circulating fluids or develop a changed reaction capacity to the same type of antigen. It seems probable, therefore, that several kinds of cells may play an important part in the synthesis of globulin and that tissue globulin functions as the mother substance or matrix from which antibodies are formed, regardless of whether they are liberated into the blood or remain at their sites of formation.

Such a matrix of globulin, if it is to function effectively both for the production of antibodies and for the preservation of the antibody mechanism itself, must be adequate qualitatively and quantitatively. Consequently, the reserves of tissue globulin must have been properly constructed during synthesis, and in sufficient amounts. Inasmuch as these arise ultimately from amino acids in the food, nutritional conditions suitable for the effective synthesis of globulin are necessary. This particular aspect of the problem will not be discussed in the present paper, since it has been considered in more detail elsewhere.[42] It is a well-known fact, however, that the production of antibodies is, as a rule, poorer in conditions of immaturity, severe malnutrition, and excessive loss of blood proteins. While the causes of this impaired capacity to form antibodies are still unknown, antibody-producing cells possessing an adequate content of tissue globulin of good quality should be capable of synthesizing as many varieties of specific antibodies as there are antigenic templates to influence their specific formation. Hektoen and Boor[39] have shown that the injection of 35 different antigens at one time engendered the simultaneous formation of 34 specific antibodies. Furthermore, Delves[43] found that the immunization of rabbits with a mixture of ten kinds of laked blood led to the simultaneous production of specific precipitins for many blood proteins and specific agglutinins and lysins for different species of blood corpuscles. These findings indicate that, under normal conditions of antibody formation, antibody synthesis depends upon

the presence within the antibody-producing cells of antigenic components, regardless of their numbers at any one time. The process may be likened to that of the printing of letters of dough for the preparation of "alphabet soup." If one compares the dough to tissue globulin, one can think of it as passing through a stamping machine with the various letters of the alphabet emerging. If, however, some of the letter molds become injured or lost, the letters will come out poorly marked or not marked at all. Furthermore, if the supply of dough runs low or deteriorates in quality because of dilution or other change, letters of poor quality will emerge.

If globulins, whether normal or specifically modified by antigen are products of cells, it becomes essential that the supply of amino acids for proper cellular functioning be constantly maintained. Furthermore, it may be highly important that the intracellular reserves of protein materials be maintained in these or in other cells of the body in order that globulin synthesis may go on uninterruptedly and even at an accelerated tempo in the presence of infection. Whipple and his collaborators[44] have shown that protein materials, particularly in the liver, constitute an important reservoir of readily mobilizable protein aggregates which are in dynamic equilibrium with the blood proteins and which can be called upon in time of need for the nourishment of the protein-depleted tissues. Schoenheimer and his associates[45] have shown also that the process of interchange of amino acids within the tissues is extremely dynamic, and that, if during the process of antibody synthesis, an amino acid (glycine) containing isotopic nitrogen is fed to a rabbit, some of the heavy nitrogen quickly appears in the antibody globulin itself. It is evident, therefore, that even during the period of antibody formation amino acids in the food may play an active part in the construction of antibody molecules.

This evidence of the active participation of amino acids of the food in antibody production re-emphasizes the problem of protein metabolism during infection. The loss of body protein during infectious disease, due to the fever, the toxemia, and the insufficient regeneration because of inadequate food intake, may be an important factor in the depletion of the protein reserves. For example, patients with typhoid fever may lose as much as 100 Gm. of body nitrogen in from eight to twelve days; the outstanding clinical fact in such patients is starvation. One may wonder in retrospect about the consequences of the low-calorie diets in the treatment of typhoid fever, particularly during those stages of the disease in which the tissues, presumably, needed an adequate supply of immune substances. It may be that the not infrequent relapses of those times were caused, in part, by the scarcity of amino acids essential for the continued production of antibodies; and perhaps the favorable effects of the Coleman-Shaffer high-calorie diet,[46] with its correspondingly higher content of amino acids, were due in some degree, to the better production of antibodies as well as to the greater caloric values and the protein-sparing effects of the carbohydrate. Coleman[47] has said concerning prognosis in typhoid fever that "when a patient is well nourished, the natural history of the disease is profoundly modified in many respects. There is evidence that complications occur less frequently. Such complications as do occur are less serious in their consequences. The immunity processes appear to be favorably influenced." He states, furthermore, that from his observations "the mortality rates in patients who were well

nourished were from 50 per cent to 75 per cent lower than in those who were partially starved.'' These observations suggest that in other subacute and chronic infectious diseases, as for example, tuberculosis, syphilis, malaria, rheumatic disease, etc., good nutrition may also facilitate recovery to a considerable degree by its action in promoting the more effective synthesis of antibody globulin.

The probability that the antibody mechanism is affected by starvation is of particular importance today as one of the problems of war. The association of war, famine, and pestilence has long been known, but the reasons for the rise of epidemics and the higher death rates from infection are still not well understood. During World War I the incidence of tuberculosis rose greatly in Germany and elsewhere, and it has been estimated that at least 800,000 persons died as a result of the food blockade, many because of infectious diseases. Part of this high death rate has been attributed to the lowered intake of protein in the diet. The possibility that depleted protein reserves may reduce the capacity of a starving person to produce antibody-globulin quickly and effectively may be of profound importance in any incipient infection which might otherwise be readily terminated. Experiments now in progress indicate very definitely that rabbits with hypoproteinemia, the result of plasmapheresis and a low protein diet, show an impaired ability to form antibodies. Such experiments suggest the tremendous importance of maintaining adequate protein reserves; the only way, obviously, is by the ingestion of sufficient amounts of protein in the diet.

The question whether there are reserves of stored protein in the tissues comparable to those of glycogen and fat is controversial, although there is but little doubt that under some conditions, as after hemorrhage or plasmapheresis and during other hypoproteinemic states, organs and tissues may contribute proteins to the blood. Nor is it known in what form or where the protein reserves are stored, although the liver undoubtedly plays an important and perhaps a predominant role. Berg[48] claimed many years ago that in well-nourished animals protein deposits could be demonstrated microscopically in the liver cells as intracellular ''protein inclusions''; in starving animals these inclusions disappeared and the cells became smaller. Furthermore, Berg and Cahn-Bronner[49] showed that, when starved animals (salamanders and rabbits) were fed a mixture of amino acids obtained from enzymatic hydrolysis of beef flesh, these inclusions reappeared in the liver cells within a few hours and were indistinguishable from those seen after ordinary protein feeding. They concluded, therefore, that these represented a phase of protein storage. Elman and Heifetz[50] showed, furthermore, in dogs kept on a low-protein diet, that as the albumin values of the blood fell, there was a concomitant decrease in the amount of stainable cytoplasm in the hepatic cells. The latter became more vacuolated and the water content of the liver as a whole increased.

There is no conclusive evidence as yet, however, as to the chemical state in which protein is stored in the liver. Luck[51] maintained two groups of rats on high- and low-protein diets, respectively, and analyzed the livers quantitatively for evidence of protein storage. He found that in the high-protein group there was an increased amount of protein per unit weight of tissue but that all the protein fractions were increased about proportionally when compared with the

similar fractions in the low-protein group. Addis and his associates,[52] in quantitative studies on the effects of fasting in rats upon protein storage, found that the liver in seven days lost about 40 per cent of its total protein, thus indicating, also, the importance of this organ as a storehouse or way station in protein metabolism. Whipple and his collaborators have demonstrated by physiologic methods the important part played by stored proteins in the bodily economy as a "reserve against adversity in the sense that it can be circumspectly depleted without apparent injury to the body." Whipple suggests that intracellular proteins may exist in three forms, viz., labile reserve protein, dispensable reserve protein, and indispensable fixed protein, and that, because of the existence of a dynamic equilibrium between the fluids and the tissues, there is an "easy exchange between the plasma protein and the labile protein stores of the body cells." Under conditions of anemia, plasma depletion, or protein fasting even the dispensable reserve proteins may also be drawn upon to some extent. The indispensable fixed proteins, however, cannot be removed unless the cells are seriously damaged or destroyed. It is of interest, furthermore, that when the "stored" proteins are contributed to the blood stream under the above conditions, the most labile protein reserves seem to be those with larger molecules. Thus Berger[53] has shown that after the injection of a foreign protein into rabbits, the resulting hyperproteinemia occurs primarily (in about four days) as a hyperglobulinemia with an associated hypoalbuminemia. The albumin levels rise later, usually in from sixty to one hundred and twenty days. Similarly, after severe blood loss in normal animals there is an increase in normal globulin in the blood, whereas, in immunized ones antibody globulin is quickly liberated into the blood, as seen in the anamnestic response after severe hemorrhage.

The facts cited in this brief review of the problem of antibody production and the anamnestic reaction suggest that antigenic components may persist within antibody-producing cells for considerable periods of time after injection or vaccination and thus are able to function as templates when later stimuli cause an acceleration of antibody production. Furthermore, there is evidence that antigenic templates which were effective earlier in the process of antibody formation tend to disappear or to become changed with time so that they lose their ability to influence specifically the later synthesis of globulin. Further studies of the anamnestic phenomenon should throw more light upon many more important aspects of the general problem of antibody production.

SUMMARY AND CONCLUSIONS

The antibody-producing mechanism comprises a widely distributed system of cells, including macrophages, fibroblasts, and possibly even endothelial cells and to some extent epidermal cells, all of which may have the potentiality in varying degrees of synthesizing globulin. Wherever this material is formed, antigenic "templates" may, if present at the sites of synthesis, so influence the structural pattern of the globulin molecules as to confer specific properties upon them. Some of these molecules may pass into the extracellular fluids as antibodies and remain there for variable periods of time; others may persist within their cells of formation. The persistence of this antibody matrix for months or years may explain both the acquisition of acquired resistance inde-

pendently of any considerable amounts of antibodies in the blood as well as many of the tissue reactivities which are commonly regarded as allergic phenomena. The effectiveness of globulin synthesis, and consequently, of antibody production will be determined by the quality and quantity of globulin reserves continuously available. These, in turn, are dependent ultimately upon an adequate intake of amino acids in the food. Diet, therefore, will determine to a large degree both the effective production of antibody globulin and the potential reserve capacity of the antibody mechanism.

REFERENCES

1. Wells, H. Gideon: The Chemical Aspects of Immunity, New York, 1929, The Chemical Catalog Company, page 109.
2. Zinsser, Hans: Resistance to Infectious Disease, ed. 4, New York, 1931, The Macmillan Company, page 133.
3. Heidelberger, Michael: The Chemistry of the Amino Acids, Carl L. A. Schmidt, Editor, Springfield, 1938, Charles C Thomas Company, Relation of Proteins to Immunity, page 959.
4. Marrack, John R.: Immunochemistry, Ann. Rev. Biochem. **11**: 629, 1942.
5. Breinl, Friedrich, and Haurowitz, Felix: Chemische Untersuchung des Präzipitates aus Hämoglobin und Anti-hämoglobin-Serum und Bemerkungen über die Natur der Antikörper, Ztschr. f. physiol. Chem. **181**: 45, 1930.
6. Alexander, Jerome: Some Intracellular Aspects of Life and Disease, Protoplasma **14**: 296, 1931.
7. Mudd, Stuart: A Hypothetical Mechanism of Antibody Formation, J. Immunol. **23**: 423, 1932.
8. Pauling, Linus: Theory of Structure and Process of Formation of Antibodies, J. Am. Chem. Soc. **62**: 2643, 1940.
9. Pauling, Linus, and Campbell, Dan H.: The Production of Antibodies in Vitro, Science **95**: 440, 1942.
10. Pauling, Linus, and Campbell, Dan H.: The Manufacture of Antibodies in Vitro, J. Exper. Med. **76**: 211, 1942.
11. Reimann, Hobart A., Medes, Grace, and Fisher, Luther: The Origin of Blood Proteins, Folia haemat. **52**: 187, 1934.
12. Downey, Hal, and Weidenreich, Franz: Ueber die Bildung der Lymphocyten in Lymphdrüsen und Milz, Arch. f. mikr. Anat. **80**: 306, 1912.
13. Sabin, Florence R.: Cellular Reactions to a Dye-Protein With a Concept of the Mechanism of Antibody Formation, J. Exper. Med. **70**: 67, 1939.
14. Landsteiner, Karl, and Parker, Raymond C.: Serological Tests for Homologous Serum Proteins in Tissue Cultures Maintained on a Foreign Medium, J. Exper. Med. **71**: 231, 1940.
15. Besredka, A.: Local Immunization, Baltimore, 1927, Williams & Wilkins Company.
16. Kahn, Reuben L.: Tissue Reactions in Immunity. XIV. The Specific Reacting Capacities of Different Tissues of an Immunized Animal, Science **79**: 172, 1934.
17. Seegal, Beatrice C., and Seegal, David: Local Organ Hypersensitiveness. VII. Demonstration of Agglutinins in Tissues of the Rabbit Eye Following Immunization With E. typhi Vaccine, Proc. Soc. Exper. Biol. & Med. **31**: 437, 1934.
18. Dienes, Louis, and Mallory, Tracy B.: Histological Studies of Hypersensitive Reactions, Am. J. Path. **8**: 689, 1932.
19. Walsh, Theodore E., and Cannon, Paul R.: Immunization of the Upper Respiratory Tract, Arch. Otolaryngol. **20**: 820, 1934.
20. Walsh, Theodore E., and Cannon, Paul R.: Immunization of the Respiratory Tract: A Comparative Study of the Antibody Content of the Respiratory and Other Tissues Following Active, Passive and Regional Immunization, J. Immunol. **35**: 31, 1938.
21. Conradi, H., and Bieling, R.: Ueber Fehlerquellen der Gruber-Widalschen Reaktion, Deutsche med. Wchnschr. **42**: 1280, 1916.
22. Dieudonné, A.: Steigerung der Agglutininbildung durch nicht-spezifische Stoffe, Med. Klin. **2**: 575, 1906.
23. Fleckseder, Rudolf: Ausschwemmung von Typhus agglutinin durch Fieber verschiedener Herkunft, Wien, klin. Wchnschr. **29**: 637, 1916.
24. Weil, E., and Felix, A.: Ueber die Beziehungen der Gruber-Widalschen Reaktion zum Fleckfieber, Wien. klin. Wchnschr. **29**: 974, 1916.
25. Dreyer, Georges, and Inman, A. C.: The Agglutination Curve and Its Importance in the Diagnosis of Typhoid and Paratyphoid Fevers in Inoculated Persons, Lancet **1**: 365, 1917.
26. Baehr, George: Agglutination in Typhus Fever, J. Infect. Dis. **21**: 21, 1917.

SIMPSON MEMORIAL INSTITUTE
ANN ARBOR, MICH.

27. Foord, Alvin G., and Forsyth, Anna: The Agglutinin Content of the Blood Following Typhoid and Paratyphoid Immunization, Am. J. Clin. Path. 3: 333, 1932.
28. Kirstein, Fritz: Steigerung der Gruber-Widalschen Reaktion durch Vakzinevirus, Deutsch med. Wchnschr. 43: 325, 1917.
29. Obermeyer, E., and Pick, E. P.: Beiträge zur Kenntnis der Präzipitinbildung, Wien. klin. Wchnschr. 17: 265, 1904.
30. Hektoen, L.: Precipitin-Production in Allergic Rabbits, J. Infect. Dis. 21: 279, 1917.
31. Bieling, R.: Untersuchungen Ueber die veränderte Agglutininbildung mit Ruhrbacillen vorbehandelter Kaninchen, Ztschr. f. Immunitätsforsch. u. exper. Therap. 28: 246, 1919.
32. Mackenzie, G. M., and Frühbauer, Emily: The Anamnestic Reaction: Response of Previously Immunized Animals to Heterologous Antigens, Proc. Soc. Exper. Biol. & Med. 24: 419, 1927.
33. Hektoen, Ludvig, and Welker, William H.: Renewal of Multiple Precipitin Production on Injection of One Antigen in Rabbits Successively Immunized With Many Antigens, Science 86: 592, 1937.
34. Topley, W. W. C.: An Outline of Immunity, Baltimore, 1933, William Wood and Company, page 183.
35. Bigler, John A., and Werner, Marie: Active Immunization Against Tetanus and Diphtheria in Infants and Children, J. A. M. A. 116: 2355, 1941.
36. Jones, F. G., and Moss, James M.: The Antitoxic Titers of Human Subjects Following Immunization With Combined Diphtheria and Tetanus Toxoids, Alum Precipitated, J. Immunol. 33: 173, 1937.
37. Jones, F. G., and Moss, James M.: Studies on Tetanus Toxoid. II. The Response of Human Subjects to an Injection of Tetanus Toxoid or Tetanus Alum Precipitated Toxoid One Year After Immunization, J. Immunol. 33: 183, 1937.
38. Jones, F. G., and Moss, James M.: Combined Diphtheria and Tetanus Toxoid, Alum Precipitated, J. Lab. & Clin. Med. 24: 512, 1939.
39. Hektoen, Ludvig, and Boor, Alden K.: Simultaneous Multiple Immunization, J. Infect. Dis. 48: 588, 1931.
40. Cecil, Russell L.: Nonspecific Protein Therapy, J. A. M. A. 105: 1846, 1935.
41. O'Leary, Paul A.: Nonspecific Treatment of Syphilis, J. A. M. A. 110: 42, 1938.
42. Cannon, Paul R.: Antibodies and the Protein Reserves, J. Immunol. 44: 107, 1942.
43. Delves, Edna: Simultaneous Immunization With a Mixture of Ten Kinds of Laked Blood, J. Infect. Dis. 57: 61, 1935.
44. Madden, Sidney C., and Whipple, George H.: Plasma Proteins: Their Source, Production and Utilization, Physiol. Rev. 20: 194, 1940.
45. Schoenheimer, Rudolf: The Dynamic State of Body Constituents, Cambridge, Mass., 1942, Harvard University Press.
46. Shaffer, Philip A., and Coleman, Warren: Protein Metabolism in Typhoid Fever, Arch. Int. Med. 4: 538, 1909.
47. Coleman, Warren: Typhoid Fever, Nelson Loose-Leaf Living Medicine, New York, 1920, Thomas Nelson and Sons, Vol. 1, page 175.
48. Berg, W.: Ueber den mikroskopischen Nachweiss der Eiweiszspeicherung in der Leber, Biochem. Ztschr. 61: 428, 1914.
49. Berg, W., and Cahn-Bronner, C.: Ueber den mikroskopischen Nachweiss der Eiweiszspeicherung in der Leber nach Verfütterung von Aminosäuren, Biochem. Ztschr. 61: 434, 1914.
50. Elman, Robert, and Heifetz, Carl L.: Experimental Hypoalbuminemia. Its Effect on the Morphology, Function and Protein and Water Content of the Liver, J. Exper. Med. 73: 417, 1941.
51. Luck, James Murray: Liver Proteins. I. The Question of Protein Storage, J. Biol. Chem. 115: 491, 1936.
52. Addis, T., Poo, L. J., and Lew, W.: The Quantities of Protein Lost by the Various Organs and Tissues of the Body During a Fast, J. Biol. Chem. 115: 111, 1936.
53. Berger, Wilhelm: Ueber die Hyperproteinämie nach Eiweiszinjektionen. Ein experimenteller Beitrag zur Pathologie des Serumproteins und sur Proteinkörpertherapie, Ztschr. f. d. ges. exper. Med. 28: 1, 1922.

CLINICAL AND EXPERIMENTAL

SARCOIDOSIS (BOECK-BESNIER-SCHAUMANN DISEASE) AS CAUSE OF A PITUITARY SYNDROME*

E. J. KRAUS, M.D., PEORIA, ILL.

COMPARED with other anatomic lesions, the involvement of the pituitary gland by sarcoidosis represents a rare event. According to a recent review on this disease by Longcope,[1] only six cases of pituitary involvement seem to have been published, all accompanied by diabetes insipidus.

The case represented in this paper differs from previous cases in that the pituitary region showed particular findings which might not have been described yet in this combination.

Today we know that sarcoidosis or Boeck's sarcoid is identical with lupus pernio described by Besnier, and that uveoparotid fever represents only a particular manifestation of the same disease. It is a chronic disease of long duration, usually characterized by a mild, often feverless, course, a tendency to exacerbation and refractory behavior to treatment, and often shows spontaneous recession. The underlying process is a granuloma with a certain resemblance to that of tuberculosis. Sarcoidosis is not identical with tuberculosis, but is often combined with it. The most frequent organs attacked are the lungs, lymph nodes, skin, spleen, liver, and eye; however, not many organs escape being involved by the granuloma. Sarcoidosis with especial involvement of the skeleton has been described erroneously by Jüngling,[2] a German author, as osteitis tuberculosa cystica multiplex.

The granulation tissue in sarcoidosis has no tendency to undergo caseation and is characterized by nodules consisting of epithelioid cells, lymphocytes, and giant cells with peculiar calcified inclusions. These nodules are frequently surrounded by a wall of hyalinized fibrous tissue. They are not prone to coalesce but remain separate though often closely approximated. The etiology of the disease is not known; transmission to animals has not been successful so far. Among the laboratory findings, one of the most significant is hyperglobulinemia.

CASE REPORT

The following case is that of a woman, aged 41 years, a teacher by occupation. Her menstruation began at 15 years of age and continued regularly for two years, the periods lasting three days. Then the menses disappeared without discernible cause, after which she bled from the nose quite regularly for two or three days every month. Prior to her death the epistaxis was so heavy that it had to be stopped at the hospital.

*From the Department of Pathology at St. Francis Hospital, Peoria.
Received for publication, May 20, 1942.

The patient gave a history of previous apical tuberculosis. She was anemic for about ten years. She died shortly after admission, her temperature having risen to 104° F.

The clinical diagnosis was hypochromic anemia, thrombocytopenia, hypoplasia of the genitals, and polyuria.

Fig. 1.—Sagittal section through the skull base, showing the dorsum sellae strikingly high and prominent.

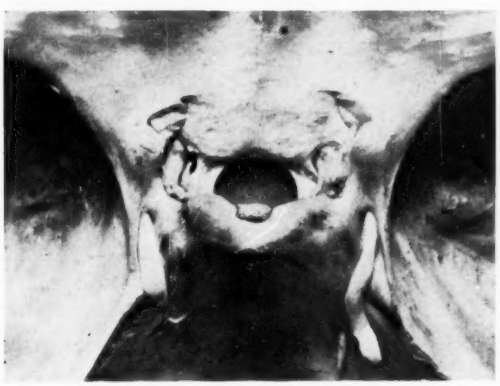


Fig. 2.—Aspect of the cranial base, showing the atrophic pituitary gland lying deep in the sella with the flattened pituitary stalk running over the abnormally high dorsum sellae.

Autopsy.—The body was 149 cm. in length and 46 kg. in weight, proportional in its dimensions. Panniculus adiposus measured 3 cm. over the abdomen, 1 cm. over the external bicipital sulcus, and 3 cm. over the fossa ovalis. The skin was without particular pigmentation. The head hair was brown and thinner than normal; the axillae, were without hair; the pubic hair was blonde, scanty, and downy; and the trunk and extremities were hairless. The breasts were small, flat, and poor in fat tissue; the nipples were not prominent; labia majora and labia minora were small.

The skull measured 17 by 14.5 cm. in diameter and up to 0.4 cm. in thickness, with a circumference of 50.5 cm. Brain and meninges were grossly without particular lesions. The pituitary gland on its cranial surface was excavated and lay deep in the sella; the dorsum sellae was unusually high and bent slightly forward. The pituitary stalk which passed over the prominent edge of the dorsum sellae was lengthened, flattened, and whitish in color (Figs. 1 and 2).

The nasal cavity was filled with coagulated blood. The thyroid was small and atrophic. In the left upper lobe of the lungs there was a deep transverse scar with neighboring bronchiectasis. The left apex contained a slaty indurated area, about 2 cm. in diameter, and two smaller calcified foci. There were fibrous adhesions of the apical pleura. Both lungs were

firmer than normal and had reduced air content; the surface in many parts was rough and showed numerous tiny grayish nodules closely compacted. In the left lower lobe a fine network of scar tissue was found in some places. The lobes of both lungs were fused together by fibrous adhesions. In other parts the pleura showed ecchymoses, as did the epicardium. The myocardium, the heart valves, and the aorta did not reveal essential changes. The bronchopulmonary and tracheobronchial lymph nodes were indurated, anthracotic, and contained a few calcified foci. The mediastinal, abdominal, and retroperitoneal lymph nodes were enlarged, especially the perigastric, peripancreatic, and para-aortal ones, which appeared to be about 2 by 3 cm. in diameter, while the iliac, hypogastric, internal inguinal, and mesenteric lymph nodes were only the size of a bean. They were firm in consistency and permeated by grayish-white nodules the size of millet seeds, the nodules being closely approximated in many places.

The spleen weighed 500 Gm., showed fibrous adhesions, and on its cut surface were many miliary nodules almost indistinguishable from the follicles. The liver, partially covered by fibrous adhesions and weighing 1,700 Gm., was light yellowish-brown in color and indurated, offering the picture of a moderate degree of cirrhosis. The gall bladder was shrunken and contained a cholesterol-pigment calcium stone. The pancreas appeared to be normal. The adrenals were small. The kidneys together weighed 200 Gm. They were firm and light yellow in color.

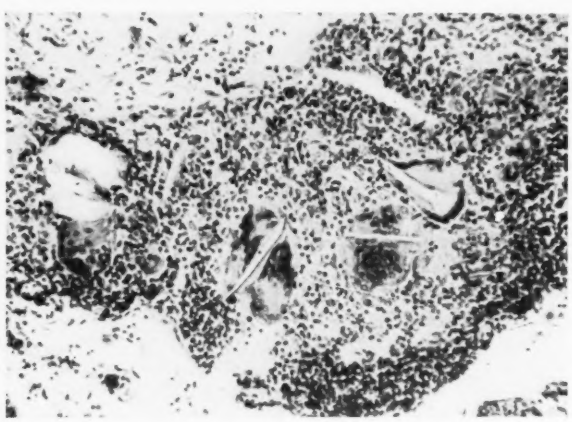


Fig. 3.—One nodule after decalcification, showing foreign body giant cells surrounding the site of the inclusions.

The uterus was small, measuring 5 by 3 cm. The corpus wall measured up to 0.5 cm. in thickness. The tubes were 11 and 12 cm., respectively, in length and very thin. The ovaries were small and shrunken, the vagina was narrow, measuring 6.5 cm. in circumference.

The femurs, ribs, sternum, and vertebras showed signs of osteoporosis. Two-thirds of the marrow in the femur consisted of light gray-red myeloid tissue while one-third was made up of fat tissue.

Anatomical Diagnosis.—Abnormal height of the dorsum sellae with compression, extension, and atrophy of the pituitary stalk; atrophy of the pituitary gland; hypotrichosis; atrophy of the ovaries, uterus, tubes, and labia pudenda; atrophy of the breasts; atrophy of thyroid and adrenals (hypopituitarism); old tuberculosis of the lung apices with fibrous adhesive pleuritis; circumscribed bronchiectasis of the left lung; peculiar granuloma (?) in all lobes of the lungs, all internal lymph nodes (apparently) and spleen; chronic splenic tumor; cirrhosis of the liver; chronic cholecystitis and cholelithiasis; osteoporosis; hemorrhage in the nasal cavity; ecchymoses in serous membranes; anemia.

Bacteriologic Examination.—Cultures on Löwenstein medium taken from spleen, lymph nodes, and bone marrow for tubercle bacilli were sterile after six weeks' observation. Guinea pigs, rabbits, and chickens inoculated with the same tissues did not show any lesions after fifty-seven days. Guinea pigs inoculated with lymph nodes, spleen, liver, and lung of these above-mentioned animals showed no pathologic lesions after fifty-three days.

Microscopic Examination.—Lungs. There are numerous miliary nodules of variable appearance. Some consist of epithelioid cells, one or a few giant cells similar to Langhans giant cells, and some round cells; frequently these are all intermingled. Other nodules are larger and contain dark violet inclusions (stained by hematoxylin), spindle, round, or rod shaped. Other nodules contain bodies suggestive of cholesterol crystals. The giant cells are intimately attached to these structures, as is typical of foreign body giant cells (Fig. 3). A number of nodules are surrounded by a thin wall of hyalinized fibrous tissue, while others appear to be fibrous also in the center. There is no tendency to conglomerate, though the nodules frequently appear close together. The final stage is a hyalinized scar containing recent nodules as well as calcified inclusions. These inclusions, which chiefly consist of calcium carbonate and calcium phosphate, also give a positive iron reaction, as shown in Turnbull blue sections. In some parts the alveolar septa are thickened, infiltrated with many lymphocytes, and contain recent as well as older and partially hyalinized nodules.

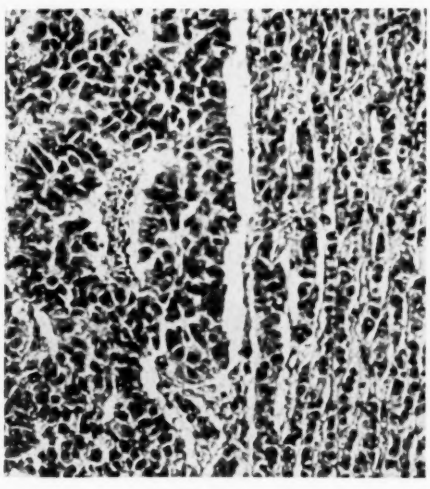


Fig. 4.

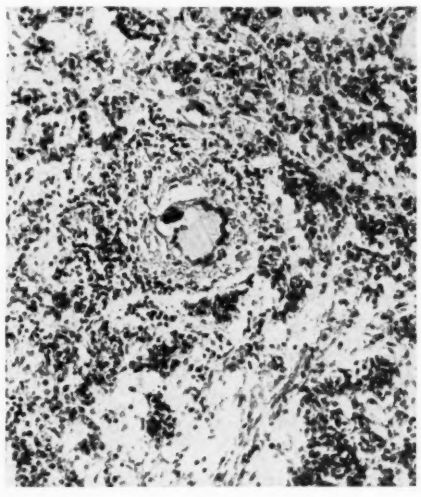


Fig. 5.

Fig. 4.—Chromophobe adenoma of the anterior lobe with adjacent parenchyma, showing atrophy by compression.

Fig. 5.—Anterior lobe of the pituitary gland, showing one nodule and fibrous atrophy of the parenchyma.

Lymph Nodes. There are many apparently very recent nodules, together with old lesions. These nodules are small, round, and well-defined accumulations of epithelioid cells with or without lymphocytes, similar to those in tuberculosis. Giant cells are sometimes present. Those with inclusions have darker stained nuclei and cytoplasm than cells without inclusions. Occasionally inclusions surrounded by a hyaline capsule are seen. In decalcified sections it becomes evident that these calcified inclusions have a particular, possibly organic, base which persists after the calcium and iron salts have been dissolved by mineral acids. By fibrosis which starts in the periphery of the nodules in the form of a hyaline shell, the granulation tissue finally passes over into scar tissue.

Liver. The liver shows a moderate degree of cirrhosis with small loose aggregations of epithelioid cells intermingled with a few lymphocytes, sometimes with giant cells, but without the afore-mentioned inclusions. Fat stains reveal a slight, irregular, fatty infiltration.

Spleen. There are microscopically small nodules, variable in appearance, with and without inclusions. There are also calcified inclusions lying free in the pulp. Small ill-defined groups of epithelioid cells apparently represent early stages of the process. The pulp appears slightly fibrotic, and the follicles are rare and atrophic.

Pituitary Gland. Serial sections of the pituitary gland cut in a sagittal plane show a marked excavation of the upper surface. In this region the cell cords of the anterior lobe appear atrophic and run parallel, as is typical of compressed parenchyma of the anterior lobe. However, the pituitary cells in other parts of the anterior lobe also appear atrophic,

being smaller than normal with the chromophilic cells evidently decreased in number. In especially atrophic parts the interstitial fibrous tissue appears thickened and hyalinized. In the middle of the anterior lobe there is a small chromophobe adenoma about 1.5 by 2 mm. in diameter (Fig. 4). The posterior lobe appears particularly small and is almost replaced by tiny nodules with giant cells and calcified inclusions. At the border of both lobes there are a few small colloid-filled follicles. The anterior lobe contains occasional little nodules in the anterior portions of the lobe (Fig. 5). The pituitary stalk is densely infiltrated with small nodules, and only microscopically small parts of the stalk are not destroyed Fig. 6). The capsule of the pituitary gland at the base of the organ is markedly thickened. The infundibulum is likewise infiltrated by tiny nodules, and there are a few on the basal surface of the optic chiasma. The other parts of the floor of the third ventricle were dissected in serial sections, but no essential lesions were found in hematoxylin-eosin stained paraffin section.

Other organs as thyroid, heart muscle, kidneys, and marrow did not show granulomatous lesions in the sections examined.

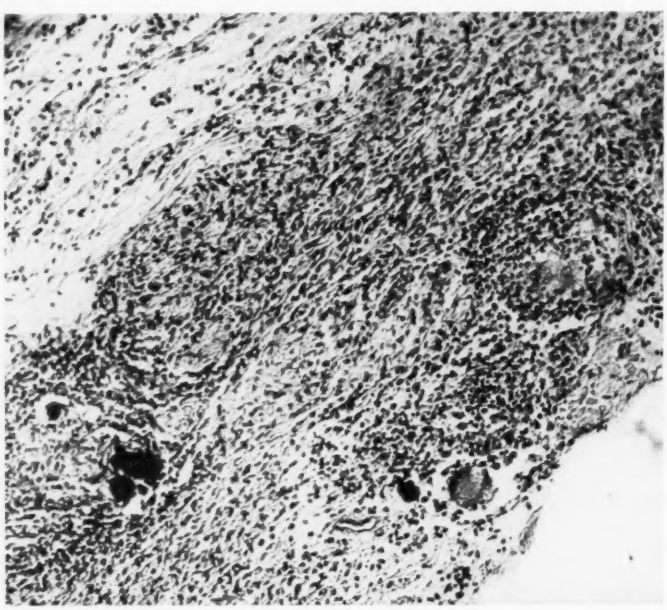


Fig. 6.—Pituitary stalk infiltrated by granuloma. The black spots represent calcified inclusions.

DISCUSSION

A case of generalized granulomatosis, together with an endocrine disease characterized by genital dystrophia, cessation of the menses, hypotrichosis, and polyuria, has been described. The granulomatosis is characterized by tiny nodules made up of epithelioid cells, lymphocytes, and giant cells differing from those of tuberculosis. These nodules show, in contrast to tubercular ones, that the lymphocytes are frequently intermingled with the epithelioid cells; they do not undergo caseation; they have a particular tendency to get surrounded by a thin hyaline capsule; they have no inclination to coalesce; and they contain peculiar inclusions consisting of an apparently organic base impregnated by calcium and iron salts, while other more rare inclusions seem to be cholesterol crystals. The inclusions give rise to foreign body giant cells, but in addition there are similar looking giant cells which reveal no relation to foreign bodies.

Inclusions, as well as giant cells, may be seen without other components of the granulation tissue, which differs from the findings in any known specific granuloma except the granuloma in sarcoidosis. This granuloma could easily be differentiated from tuberculosis not only by its particular histology as just described but also by negative animal inoculation and cultures.

The involvement of the pituitary and its stalk caused a pituitary disturbance characterized by cessation of menses (substituted, however, by monthly epistaxis), loss of body hair, and polyuria. At autopsy the hypopituitarism manifested itself by atrophy of the ovaries, uterus, tubes, outer genitals, and breasts, and by atrophy of thyroid and adrenals and marked hypotrichosis.

In addition to the destruction of the pituitary stalk and posterior lobe of the pituitary gland by the granuloma, another lesion was found that also might have played a part in the pathogenesis of the hypopituitarism. It was an unusually high and steep dorsum sellae that compressed and stretched the stalk to an abnormal length. How much the chromophobic adenoma in the anterior lobe influenced the picture is hard to say. It might have been compensatory in nature, owing to the atrophy of the anterior lobe, and thus without particular significance as to the development of the pituitary disease.

Since the patient seemed to be normal up to 17 years, with regular menstruation, the anomaly of the dorsum sellae might not have impaired to any great extent the function of the pituitary gland but the damage to the pituitary stalk might have created a "locus minoris resistentiae" where the granuloma easily established itself. On the other hand, the report of diabetes insipidus in other cases of sarcoidosis shows that an involvement of the nervous part of the hypophyseal-diencephalic system is not necessarily based on a previous lesion of this region as described here.

After the granuloma had destroyed the pituitary stalk and the infundibulum, the communication between the pituitary gland and the vegetative centers in the hypothalamus was cut off, the result of which was the atrophy of the anterior lobe. As is known the separation of pituitary gland from the hypothalamus inevitably leads to atrophy of the gland, especially the anterior lobe due apparently to the loss of nerve communications with the vegetative centers in the floor and wall of the third ventricle, and also due to the interruption of channels carrying certain products of the pituitary glands (for instance, gonadotropic hormone) to the diencephalon (Kraus).[3] In view of the long duration of the sarcoidosis it is quite possible that the involvement of the pituitary region by the granuloma reaches far back and that the cessation of the menses might have been the first manifestation of the pituitary disturbance. But it is also possible that the pressure on the pituitary stalk by the anomalous dorsum sellae induced the pituitary disease, which was later much aggravated by the destructive granuloma.

While the disturbance of the water balance certainly resulted from the functional loss of the posterior lobe and pituitary stalk, the genital dystrophia with the hypotrichosis must be referred to the marked atrophy of the anterior lobe.

The atrophy of the thyroid and adrenals also is a result of the pituitary insufficiency as is the generalized atrophy, including the osteoporosis. Since emaciation does not belong to the picture of sarcoidosis, one may suppose that

the generalized atrophy in this case (the patient weighed only 46 kg.) was pituitary in nature.

The vicarious menstruation from the nasal mucosa of many years' duration is hard to explain when one considers the ovaries were entirely atrophic. The later profuse and finally fatal character of the epistaxis might be due to the thrombocytopenia. Since this symptom is not mentioned in other cases of sarcoidosis (while eosinophilia is quite frequent) a causal connection between the sarcoidosis and the blood lesion is not probable, nor is there particular reason to assume pathogenetic relations between the thrombocytopenia and the pituitary disease.

SUMMARY

A case of sarcoidosis (Boeck-Besnier-Schaumann disease) in a woman 41 years of age has been reported. The following microscopic changes have been found characteristic of this granuloma: tiny nodules made up of epithelioid cells, giant cells, and round cells, the latter often intermingled with the epithelioid cells; no tendency to coalesce but rather to be surrounded by a hyaline-fibrous capsule; no tendency to caseation; peculiar inclusions made up of calcium and iron salts compounds deposited in an organic ground substance surrounded by giant cells; occurrence of inclusions, as well as giant cells, alone without other components of the granulation tissue, and finally, conversion into fibrous tissue.

An endocrine syndrome characterized by genital dystrophia, cessation of menses (with vicarious menstruation from the nasal mucosa), hypotrichosis, polyuria, and generalized atrophy has been shown to be caused by the granuloma which has involved the infundibulum, the pituitary stalk, and the pituitary gland itself. An anomaly of the dorsum sellae with pressure on the pituitary stalk probably caused a locus minoris resistentiae for the granuloma. A chromophobe adenoma of the anterior lobe has been interpreted as a compensatory proliferation in an otherwise atrophic organ. A thrombocytopenia causing death could not be referred either to the sarcoidosis or to the endocrine disturbance.

REFERENCES

1. Longcope, W. T.: Sarcoidosis or Besnier-Boeck-Schaumann Disease, J. A. M. A. **117**: 1321, 1941.
2. Jüngling, O.: Ostitis tuberculosa multiplex cystica, Fortschr. a.d. Geb. d. Röntgenstrahlen **27**: 375, 1919.
3. Kraus, E. J.: Die morphologischen Veränderungen der menschlichen Hypophyse nach Zerstörung der Zwischenhirnbasis bzw. des Hypophysenstiels und deren Folgen, Virchows Arch. f. path Anat. **286**: 656, 1932.

TREATMENT OF CONGESTIVE HEART FAILURE IN AMBULATORY PATIENTS WITH AN ORALLY ADMINISTERED MERCURIAL DIURETIC*

JULIUS BURSTEIN, M.D., GEORGE BROWN, M.D., AND CHARLES KLEIN, M.D.
NEW YORK, N. Y.

IN 1940 Batterman, DeGraff, and Rose reported the satisfactory use of an oral preparation of salyrgan-theophylline† in hospitalized patients suffering from congestive heart failure.[1]

It seemed desirable to test the efficacy of this preparation in the treatment of ambulatory patients with congestive heart failure who ordinarily required the frequent administration of intravenous mercurials for maintenance. This study was conducted with patients attending the Morrisania City Hospital Cardiac Clinic.

SUBJECTS, MATERIAL, AND METHODS

Nine patients were selected from a group of 20 who were being treated for congestive heart failure in the out-patient clinic. In all these patients symptoms and signs of failure were not controllable by means of digitalis and the usual oral diuretics (ammonium chloride, aminophylline) alone, but adequate diuresis was obtained by means of intravenous injections of mercurial diuretics.‡ These patients, all ambulatory, had been carried adequately, i.e., nocturnal dyspnea, edema, and weight had been controlled, with one or two weekly injections intravenously of 2 c.c. of a mercurial diuretic, for periods varying from a few months to three years. Of this group of patients, 9 were selected for treatment with the oral preparation of salyrgan-theophylline.† The other 11 patients could not be taken off intravenous mercurial therapy, since the severity of their symptoms did not permit any change of regimen.

Of the 9 patients studied, 6 were men and 3 were women. The age range was from 41 to 69 years: 4 patients suffered from both arteriosclerotic and hypertensive heart disease, 2 from arteriosclerotic heart disease, one from hypertensive heart disease, and 2 from rheumatic heart disease. The urines of all 9 patients, on repeated examinations, showed normal variability in specific gravity, and yielded no pathologic microscopic findings. Blood chemistry determinations were all normal.

For two weeks prior to the change from intravenous to oral mercurial therapy, the 9 patients reported to us, on printed instruction sheets, their urine outputs for the twenty-four-hour period immediately following the mercurial

*From the Cardiac Clinic of the Morrisania City Hospital, Bronx, N. Y.
Received for publication, May 21, 1942.

†Supplied by Department of Medical Research of the Winthrop Chemical Co., labelled S.T.O. 3813. Each tablet contains 80 mg. of salyrgan (30 mg. of mercury and 40 mg. of theophylline).

‡Mercupurin, salyrgan-theophylline. Each cubic centimeter contains the equivalent of 40 mg. of mercury.

injection, and for the twenty-four-hour period just preceding the next injection. This last figure represented the daily output without the influence of a mercurial diuretic, since the measurements were made several days after the effect of the drug had worn off. The patients were instructed to restrict their fluid intakes to 1,000 c.c. daily. They had been receiving 4 to 6 Gm. of ammonium chloride daily as long as they had been on intravenous mercurial therapy.

TABLE I

NAME	AGE	SEX	TYPE OF HEART DISEASE	WEIGHT BEFORE S.T.O.	WEIGHT AFTER S.T.O.	DAILY OUTPUT WITHOUT MERC. DIUR. (C.C.)	DAILY OUTPUT AFTER S.T.O. (C.C.)	DAILY OUTPUT AFTER INTRAV. MERC. (C.C.)	REMARKS
L. C.	60	M	Arteriosclerotic and hypertensive	207	207	1,000	1,500	2,500	Diarrhea
D. S.	49	F	Hypertensive	1-168	166	1,000	3,000	4,500	Onset of diuresis slow
				2-170	171	1,000	Not reported		Severe cramps and diarrhea
D. O.	50	M	Arteriosclerotic	1-175	175	800	1,250	2,000	Mild cramps and diarrhea
				2-181	181	1,000	1,100		No side effects
				3-182	178	1,000	2,000		Severe cramps and diarrhea
L. W.	55	M	Rheumatic and arteriosclerotic	1-161	162	1,000	1,250	5,000	No side effects
				2-153	146	1,000	4,000		No side effects
				3-151	147	750	2,300		No side effects
				4-		Not reported			
				5-		Not reported			
C. T.	41	F	Rheumatic	1-154	152	1,250	3,750	Not reported	Cramps and diarrhea
				2-152	151	1,250	2,750		Cramps and diarrhea
D. S.	67	F	Arteriosclerotic and hypertensive	159	157	750	1,000	2,000	No side effects, but did not feel as comfortable as after intravenous mercurial
S. K.	60	M	Arteriosclerotic and hypertensive	202	199	2,000	3,600	Not reported	No side effects
J. O.	69	M	Arteriosclerotic and hypertensive	142	142	1,500	1,250	Not reported	Cramps and diarrhea
A. N.	64	M	Arteriosclerotic	1-166	164	550	4,000	5,500	No side effects
				2-164	163	550	1,000		No side effects

Then, still keeping the patients on the same fluid intake, and maintaining the administration of ammonium chloride as before, the patients were given 5 tablets of oral salyrgan-theophylline immediately following breakfast on the

day of administration, in place of the intravenous mercurial injection. Again the patients were instructed to measure and report the urine output for the twenty-four-hour period immediately following the oral administration of this preparation, and again for the twenty-four-hour period before the next dose. During this study the patients were weighed just before the tablets were given and again forty-eight hours later.

The results of this study are shown in Table I.

ANALYSIS OF RESULTS

The 9 patients studied received oral salyrgan-theophylline a total of 18 times. The results were not reported three times, leaving a total of 15 reportable trials.

Of the 9 patients studied, one (one trial) showed a decrease in urinary output, about 15 per cent less than when not under the influence of a mercurial diuretic. One patient (two trials) showed no change in output. The other 7 patients (12 trials) all showed an increase in urinary output over that observed when not influenced by the administration of a mercurial diuretic, the increase ranging from 10 to 63 per cent; 7 trials showed an increase of 100 per cent or over; 5 trials, an increase of less than 100 per cent.

All 9 patients reported a greater increase in urinary output after receiving intravenous mercurial than was obtained by the use of oral salyrgan-theophylline. Of 8 measured results after intravenous injection, the range of increase was 66 to 900 per cent, with 7 showing an increase of 100 per cent or more and one an increase of 66 per cent.

Six of the 9 patients developed cramps or diarrhea or both after one to three doses of oral salyrgan-theophylline, which were severe enough to require that the medication be discontinued.

SUMMARY

Nine patients in congestive heart failure, attending an out-patient cardiac clinic, who were known to be well controlled by administration of intravenous mercurial diuretics, were given instead, an oral preparation of mercurial diuretic in a study to determine the usefulness of this medication in ambulatory patients. Seven of the 9 patients (77.7 per cent) showed an increase in urinary output after taking this preparation, as compared with their output when no mercurial diuretic was given. In no case was the increase in urinary output as great as that resulting when the usual dose of a standard mercurial diuretic was given intravenously. Side reactions, chiefly cramps and diarrhea, occurred in 6 of the 9 patients (66.7 per cent) and necessitated discontinuance of administration of the oral mercurial preparation.

REFERENCE

1. Batterman, R. C., DeGraff, A., and Rose, O. A.: Treatment of Congestive Heart Failure With an Orally Administered Mercurial Diuretic, Am. Heart J. 21: 98, 1940.

ADENYLIC ACID AND BACTERIAL GROWTH

SAUL CASPE AND A. G. OSLER*
NEW YORK, N. Y.

DIRECT bacterial infection of the living muscle tissue is not a common phenomenon. Due to its anatomic position the only avenues for muscular infection are through the lymphatics or the blood circulation. Because the latter have their own protective mechanisms, the muscle is thus usually spared from infection. In addition to the above, we believe that the chemical changes and end products occurring during muscular metabolism are inimical to bacterial growth.

In the production of experimental streptococcic arthritis in rabbits, Hadjopoulos[1] and Burbank demonstrated that, whereas the bone marrow, muscular sheaths, and even tendons were infiltrated with streptococci, there was no evidence of the invasion of the muscle tissue. Ammonia and lactic acid are known to be inimical to bacterial growth. However, their concentration in the muscle is not sufficient to inhibit the growths of certain types of bacteria.

The work of Lutwak Mann[2] showed that washed suspensions of *Esch. coli* and of other Bacteriacea dephosphorylated and deaminated adenosine triphosphoric acid, and adenylic acid. These reactions occurred with fresh suspensions as well as with those treated with toluene, and these facts have led Lutwak Mann and others to the conclusion that these reactions are due to bacterial enzymes. What effect the adenine compounds and their dephosphorylated and deaminated derivatives have upon the growth of various types of bacteria was not determined. In order to obtain this information, we devised preliminary experiments.

Our plan, therefore, is to study the effect of adenylic acid upon the growth of various types of bacteria. The usual in vitro experiments do not correspond to the conditions that obtain in normal physiologic processes of the living organism. We desire to approximate these normal body conditions. The work of Freedman and Funk[3] offered application in this connection. These investigators were able to inhibit the growth of bacteria in beef heart broth shaken with charcoal. They postulated the theory that by this procedure a new vitamin was adsorbed upon the charcoal, a vitamin necessary for bacterial growth. By this method, we are afforded an opportunity of observing bacterial growth in a media partially depleted of a bacterial vitamin, and this experimental condition might simulate the conditions occurring in certain parts of the human body.

EXPERIMENTAL

We compare the growth of *B. coli, Staphylococcus aureus, Streptococcus hemolyticus* and *viridans* on (1) starvation media; (2) beef heart infusion; (3) beef heart infusion after treatment with charcoal; (4) beef heart infusion

*Consulting chemist and bacteriologist, New York.
Received for publication, May 1, 1942.

after treatment with charcoal and addition of 0.2 per cent adenylic acid; and (5) starvation media with addition of 0.2 per cent adenylic acid.*

The inoculum for all tests is prepared as follows: Individual organisms are grown in broth culture and are seeded onto agar slants (eighteen hours' growth). The growth is washed with saline, centrifuged, and, again washed four or five times. Then a dilute suspension of the growth is inoculated into a flask containing 10 c.c. of media. The flasks are incubated for ninety-six hours. They are then centrifuged in Hopkins tubes and read. Table I gives the results of this experiment.

TABLE I*

96 HR. GROWTH	GLUCOSE SALT SOLUTION	B.H.I. (DIFCO)	B.H.I. AFTER CHARCOAL	B.H.I. AFTER CHARCOAL PLUS ADENYLIC ACID	G.S.S. PLUS ADENYLIC ACID
B. coli	No growth	B.C. –3.5B H.T. –0.065	B.C. –1.23B H.T. –0.01	No growth	No growth
Streptococcus hemolyticus	No growth	Heavy growth	Very light growth	No growth	No growth
Streptococcus viridans	No growth	Heavy growth	Very light growth	No growth	No growth
Staphylococcus aureus	No growth	H.T. –0.037	H.T. –0.01±	No growth	No growth

*B.C.—Bacteria in billions per cubic centimeter. H.T.—Height of tube. B.H.I.—Beef heart infusion. G.S.S.—Starvation media.

Using a similar procedure the growths of *B. coli* in beef heart infusion, and with 0.2 and 1 per cent adenylic acid are determined. The results are given in Table II.

TABLE II

24 HR. GROWTH	B.H.I. (DIFCO)	B.H.I. PLUS 0.2% ADENYLIC ACID	B.H.I. PLUS 1.0% ADENYLIC ACID	0.5% ADENYLIC ACID (CONTROL)
B. coli	H.T. –0.028	H.T. –0.022	H.T. –0.007+	
				0.001+ (sediment)

The concentration of adenylic acid used in our experiments is probably comparable to the content of adenine compounds of the muscle. The work of at least two workers in this field can be used as reference for such comparison.[4, 5]

It is demonstrated that adenylic acid inhibits the growth of ordinary types of bacteria.

We are indebted to Dr. L. G. Hadjopoulos for his cooperation in these experiments.

REFERENCES

1. Hadjopoulos, L. G., and Burbank, R.: Correlation of Experimental Streptococcic Arthritis in Rabbits With Chronic Rheumatoid Arthritis, J. Bone & Joint Surg. **14**: 471, 1932.
2. Lutwak Mann, C.: The Decomposition of Adenine Compounds by Bacteria, Biochem. J. **30**: 1405, 1936.
3. Freedman, L., and Funk, C.: Nutritional Factors in the Growth of Yeasts and Bacteria, J. Metabol. Research **1**: 457, 1922.
4. Bennet, D. W., and Drury, A. N.: Further Observations Relating to the Physiological Activity of Adenine Compounds, J. Physiol. **72**: 288, 1931.
5. Feinschmidt, O.: The Metabolism of Adenosine Triphosphate in Muscles, Biochem. Ztschr. **286**: 290, 1936.

474 WEST 238 STREET

*The glucose-salt mixture, which represents our starvation media, is made according to Freedman and Funk. The beef heart infusion is Difco. It is treated with 10 per cent charcoal, and the infusion is heated on a boiling water bath for ten minutes, filtered, and sterilized.

FATAL SALMONELLA INTRACRANIAL INFECTION IN AN INFANT*

Report of a Case

W. E. Bray, M.D., and J. M. Meredith, M.D.
University, Va.

CLINICALLY and bacteriologically the Salmonella group presents many problems.

Clinically, some species or types, as, for example, *S. paratyphi* or *B. paratyphosus A*, infect man only and cause prolonged fever and symptoms indistinguishable from typhoid fever; others, like *S. aertrycke*, cause chiefly gastrointestinal upsets, with sudden onset and short duration, called food poisoning, and are found in both animals and man; and still others, which rarely if ever infect man, like *S. pullorum*, which causes white diarrhea of chickens, are important only economically.[1]

Bacteriologically, there is not only variation in the cultural characteristics, such as in the fermentation of the various sugars, but there is marked variation in the antigen components and consequently in the serologic reactions. Following the work of White[2] (1926-1929) and of Kauffmann[2] (1929-1934) and of later workers[3] in antigen analysis, so many species or types have been described that Jordan and Burrows,[4] in their recent edition of their *Textbook of Bacteriology*, consider that simplification is essential.

The organism cultured from the case here reported was morphologically and culturally like one of the Salmonella group, especially *S. enteritidis*, and was agglutinated to a moderate titer with antisera of *S. aertrycke* and *S. schottmülleri*, but only very slightly with antiserum of *S. enteritidis*. Because of these findings and because of the unusual clinical picture, the culture was sent to the Salmonella Typing Station, located at the University of Kentucky, Lexington, Ky., for identification of the type of the Salmonella. Dr. P. R. Edwards, bacteriologist, Department of Animal Pathology, who has kindly given us permission to use his reports, found the culture to be *S. sandiego*, the antigenic formula of which is IV, V: eh enz_{15}.

S. sandiego was first described by Kauffmann,[5] who studied the *S. chester* group and, on the basis of the structure of the H antigen, particularly the β phase, divided the group into two serologic types: *S. chester* and *S. sandiego*.

To the *S. sandiego* type belong:

1. Two original cultures isolated by Professor K. F. Meyer of San Francisco from a case of food poisoning in an outbreak at the San Louis Rey Mission,

*From the Departments of Clinical Pathology and of Neurological Surgery, University of Virginia Hospital and School of Medicine.
Received for publication, May 27, 1942.

where two hundred persons suffered from severe abdominal pain accompanied by vomiting and diarrhea. Most patients recovered within one to two days, but some were ill for ten days or longer.

2. An old laboratory culture, No. 1254, isolated in 1929 in Denmark and mistaken for *S. reading*.

3. Culture CB 42/2, isolated from a healthy pig by Professor E. Hormaeche of Montevideo, Uruguay.

4. Culture b641, obtained from a human being by Professor Hormaeche.

5. Culture 123, obtained by Dr. P. R. Edwards, Lexington, Ky.

The antigenic formulas for these strains are given in Table I, to which we have added the formula for our strain, as determined by Dr. Edwards.

TABLE I

STRAIN	ANTIGEN FORMULA	
Original	IV, XII	eh enz_{15}
1254	IV, XII	eh enz_{15}
CB 42/2	IV, V, XII	eh enz_{15}
641	IV, V, XII	eh enz_{15}
123	IV, V, XII	eh enz_{15}
UVa	IV, V	eh enz_{15}

Dr. Edwards[6] has typed 17 cultures of *S. sandiego:* four from animals, one from sewage, and 12 from man.

The most unusual feature of the following case is the localization of the infection in the subdural space, forming an abscess. The Salmonella infections tend to localize in the bones, joints, and other tissues,[7] and certain members of the Salmonella group, notably *S. enteritidis* or Gärtner's bacillus, have frequently been reported as causing meningitis.[8]

PRELIMINARY NOTE

Baby R. M., Jr., a white male infant, aged 4½ months, University of Virginia History No. 163058, was admitted April 2, 1941, and died April 16, 1941.

The child was born at the University of Virginia Hospital on Nov. 12, 1940, and remained until Dec. 31, 1940. He was a premature child (seven months), forceps being required for delivery. His birth weight was 2,185 Gm. When discharged (Dec. 31, 1940) he was in good condition (weight 2,975 Gm.), although his head was observed to be large, even at that time. At birth the circumference of the head was 35.5 cm.; normally it is 34.7 cm.

The child was next seen on April 1, 1941 (aged 4½ months) in the pediatric out-patient department. He appeared to be very ill. His temperature was 102.2° F. (rectal). The circumference of his head was 53.2 cm. (normal, 40.5 cm.), it being tremendously enlarged. He had vomited for two days previous to his visit to the clinic.

Examination at that time (April 1, 1941) revealed what appeared to be marked hydrocephalus (Fig. 1). The fontanelles were very large and the cranial sutures were widely separated. The child gazed downward constantly in a typically hydrocephalic appearance.

REPORT OF CASE

The following day (April 2) the child was admitted to the hospital on the neurosurgical service for study and possible operation, with a temperature of 103.2° F. (rectal). Each lateral angle of the anterior fontanelle was aspirated and very sanguineous, somewhat purulent, fluid was obtained. This fluid contained 46,900 R.B.C., and 1,980 W.B.C. per cubic millimeter. The following day (April 3) the erythrocytes of the aspirated fluid were diminished in number, but the white blood cells were considerably increased (27,300 R.B.C.; 8,400 W.B.C., 84 per

cent P.M.N.); one week later (April 10) the fluid was very cloudy and contained only 680 R.B.C. but 30,150 W.B.C. per cubic millimeter. Two days before death (April 14) the fluid aspirated through the anterior fontanelle contained 42,600 W.B.C. per cubic millimeter and appeared frankly purulent, with no gross blood discernible.

Examination of the fluid aspirated through the fontanelle on two occasions, for the presence of tumor cells was negative (hematoxylin and eosin stain of centrifuged specimen).

Fig. 1.—Photograph of the patient (twenty-four hours after the first aspiration of fluid through the anterior fontanelle) taken on the day after admission to the hospital (April 3, 1941). Note the very large head, the engorged scalp veins, and the typical downward gaze of the eyeballs so often seen in hydrocephalic infants.

The blood and ventricular fluid Wassermann reactions were negative. Urinalysis revealed many large granular pseudocasts but no red blood cells and no albumin or sugar. An occasional white blood cell per high-power field was seen. Blood counts are shown in Table II.

TABLE II

DATE	HEMOGLOBIN (%)	HEMOGLOBIN (GM. %)	R.B.C. (MILLIONS)	W.B.C.
4/ 3/41 (Day after admission)	62	9.5	3.03	9,300 P.M.N. 28%, Lymph 67%, Mono. 5%
4/ 6/41	65	10.0	5.08	21,000
4/ 8/41			4.16	19,200
4/11/41			3.84	11,200
4/14/41			3.16	34,500

Bacteriologic Examination of Removed Intraventricular or Subdural Fluid.—This was done on several occasions. It soon became evident that none of the ordinary pyogenic bacteria were the cause of the infection. A Salmonella-like

organism was isolated, designated *Salmonella sandiego*, constituting the first instance of an intracranial infection from this organism in our experience.

Course in the Hospital.—The child's condition, as might be expected, was precarious at all times. He died two weeks after admission. His terminal temperature was 101° F (rectal), the temperature readings fluctuating widely, the highest reading being 105.2° F. (rectal) the day after admission. The anterior fontanelle remained very tense at all times except immediately after aspiration. Convulsions occasionally occurred. Sulfathiazole, sulfapyridine, and transfusions of blood were given. Cheyne-Stokes respiration and cyanosis were frequently observed. The immediate cause of death was respiratory failure incident to the overwhelming intracranial infection. A post-mortem examination could not be obtained.

DISCUSSION

An intracranial infection from the organism identified in this case (*S. sandiego*) is very rare. It is possible that the child had a primary (congenital) hydrocephalus on which was engrafted the Salmonella infection, since the child's head was enlarged at birth. Interest also centers on the exact location of the intracranial infection. Was it primarily intraventricular or subdural? The impression of one of us (J. M. M.) who observed the child daily in the ward was that it was primarily subdural, although this can never be proved conclusively since no burr openings were made over the cerebral hemispheres and no post-mortem examination was permitted. It is also quite conceivable that the intracranial involvement began as a purulent meningitis from a primary infection elsewhere in the body (gastrointestinal tract) or from a blood stream infection, proceeding on to gross intraventricular infection (purulent ependymitis) and subdural infection as well.

EPIDEMIOLOGY

Efforts were made to determine the manner in which the infant might have obtained such an unusual infection. Dr. T. S. Englar, Director of the Joint Health Department of the County of Albemarle (Va.) and the City of Charlottesville, kindly instituted a survey of the child's home environment in a nearby country district. His department found that the child had not been breast fed. The mother had been very careful to boil all milk before feeding the baby.

The baby's diet had consisted of boiled milk with Karo syrup added, as well as orange juice and water. He had been fed entirely by bottle and nipple, which were consistently washed and boiled. The milk was usually boiled each morning and night.

The baby's mother continually complained of feeling bad, with headache and nausea, frequently not eating at mealtimes. She did not complain of diarrhea or dysentery at any time so far as is known.

The water supply was from two open springs. Samples of water from these springs were examined in the laboratory of the Department of Preventive Medicine and Bacteriology, University of Virginia, Dr. G. M. Lawson, director. The sample from one spring showed a count of 18 colonies per cubic centimeter, with *B. coli* and *B. aerogenes* confirmed in three tubes of 10 c.c. each; from the

other spring, 38 colonies per cubic centimeter, with *B. aerogenes* confirmed in one tube of 1 c.c., and two tubes of 10 c.c.

The sewage was disposed of through a pit-type toilet located so as not to contaminate the water supply.

The home milk supply was from two cows. One had been sold before the health department survey was made; bacteriologic analysis of milk from the udders of the remaining cow (collected directly into a sterile bottle) showed a count of 56,000 with no lactose fermenters.

SUMMARY

A fatal intracranial infection due to *Salmonella sandiego*, an uncommon type of the genus, is reported in an infant.

A list of the reported cultures of this type, with their antigenic formulas, is given.

This appears to be the first report of this organism since the original description of the type.

Addenda.—Since this paper was prepared, another case of infection with *S. sandiego* in which localization of the infection occurred in the right kidney, and apparently also in the degenerated and necrotic areas of a large uterine fibroid, has been treated in the University of Virginia Hospital. The course of this illness, extending over a period of seventy-six days, was septic in character with blood stream infection, and recovery began apparently only after the infected foci were removed by surgery.

REFERENCES

1. Havens, Leon C.: The Bacteriology of Typhoid, Salmonella and Dysentery Infections and Carrier States, New York, 1935, Commonwealth Fund, page 96.
2. Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunology, ed. 2, Baltimore, 1936, William Wood and Co., page 546.
3. Edwards, P. R.: J. Bact. **32**: 259, 1936.
   Edwards, P. R., Bruner, D. W., and Rubin, H. L.: Proc. Soc. Exper. Biol. & Med. **44**: 395, 1940.
   Bruner, D. W., and Edwards, P. R.: Am. J. Hyg. **34**: 82, 1941.
   Kauffmann, F.: J. Bact. **41**: 126, 1941.
4. Jordan, Edwin O., and Burrows, William: Text Book of Bacteriology, Philadelphia, 1942, W. B. Saunders Co., page 350.
5. Kauffmann, F.: Acta path. et microbiol, Scandinav. **17**: 429, 1940.
6. Personal communication.
7. Goulder, N. E., Kingsland, Margaret F., and Janeway, C. A.: New England J. Med. **226**: 127, 1942.
8. Meyer-Rohn, J.: Deutsche med. Wchnschr. **66**: 1193, 1940. (Gärtner bacillus.)
   Wengeler, J.: Med. Klin. **36**: 1217, 1940. (Gärtner bacillus.)
   Guthrie, K. J., and Anderson, T.: Brit. M. J. **1**: 193, 1941. (Gärtner bacillus and meningococcus.)
   Wilsdon, K. F., and Gibson, A.: Lancet **1**: 665, 1941. (*S. london.*)

EFFECT OF INJECTION OF TISSUE EXTRACTS ON THE NUMBER OF BLOOD PLATELETS*

ALFRED UHLEIN, M.D., ROCHESTER, MINN.

THIS investigation was stimulated by experiments[3, 19-21] which indicated that a thrombocytolytic agent, "thrombocytopen," can be extracted from spleens of patients who have idiopathic hemorrhagic purpura and was undertaken at the suggestion of Dr. H. Z. Giffin and Dr. J. deJ. Pemberton. Some reports in the literature demonstrated that a substance can be isolated which not only decreases the number of circulating blood platelets of animals,[2, 18, 19] but even alters the normal configuration of the megakaryocytes when cultured in vitro.[14-17] The thrombocytolytic substance apparently reduces the number of platelets, but experimentally, it does not produce purpura. During the progress of this study, several investigators[1, 4, 10, 13] reported their inability to isolate thrombocytopen, while others confirmed its presence.[5, 7, 12]

METHOD

For the purpose of investigation, extracts of 28 spleens, together with normal hepatic and lymphoid tissue from one patient, were prepared. Thirteen spleens were from patients who had idiopathic hemorrhagic purpura, five from patients who had hemolytic icterus, five from those who had Banti's disease, and one from a patient who had Hodgkin's disease. There were four normal spleens. All but three of the spleens were obtained from the operating room, and each was prepared for extraction within thirty minutes after splenectomy. Three spleens and the hepatic and lymphoid tissues were obtained within an hour and a half after death.

The tissues were extracted according to the procedure outlined by Troland and Lee.[21] Briefly, each tissue was ground in a medium-fine meat grinder and weighed. It was then placed in a jar containing acetone, the volume of which was twice the weight of the tissue. The container was sealed and permitted to remain at room temperature for about four weeks, gently agitated at least once a day. Three spleens were extracted in commercial acetone, while all other tissues were placed in reagent acetone. After extraction the mixture was filtered and centrifuged. The acetone then was removed by distillation. The residue was brown and gummy. It was mixed with a sufficient amount of distilled water to make 1 c.c. represent 1 Gm. of tissue. This aqueous mixture again was filtered and the filtrate was placed in the refrigerator where it remained for about two

*From the Mayo Clinic, Rochester.

Abridgment of thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

Work done in the Institute of Experimental Medicine, the Mayo Foundation, Rochester.
Received for publication, May 25, 1942.

weeks. If a precipitate formed, gentle agitation and passage through a Zeiss filter cleared the filtrate. A preservative was not added.*

At first, experiments were conducted on rabbits that averaged 2 kg. After a few experiments, however, rabbits were rejected because it was difficult to obtain control platelet counts from them, and rats weighing 200 Gm. (Wistar strain) were used. Control platelet counts were carried out for from one to three days prior to injection of the extract. Usually 1 c.c. of the extracted material, the equivalent of 1 Gm. of tissue, was injected intravenously; however, as much as 20 c.c. were given. Subcutaneous and intraperitoneal injections also were tried in a few instances. Following intravenous injection the number of platelets was determined at hourly intervals for the first six hours, and thereafter, every six hours until no further variation in the number of platelets was noted. Each animal received only one injection.

The method used for counting the platelets differed slightly from that of Troland and Lee.[21] A 2 per cent solution of sodium citrate in normal saline solution, as described by Ottenberg and Rosenthal but modified by the Division of Clinical Pathology of the Mayo Clinic, was used instead of the Rees-Ecker solution, as the latter diluent contained too many platelet-like artifacts. The solution of sodium citrate contained 2.26 Gm. of sodium citrate, 1.8 Gm. of sodium chloride, and 220 c.c. of distilled water. The solution was thrice filtered into a sterile flask. The diluent was always freshly prepared and was used at room temperature.

Blood was obtained by cutaneous puncture of the ear of the animal. The first few drops were discarded,[8, 9] and specimens were not collected unless the blood flowed freely without compression of the ear. However, gentle compression of the ear was at times necessary after injection of the extract because the blood flow was decreased; the cause of this was not apparent. The blood was drawn up to the 0.5 mark in an erythrocyte pipette and diluted to 1.1 with the solution of sodium citrate. This mixture was agitated gently by hand for three minutes. Following a five-minute quiescent period a third of the contents in the bulb of the pipette was expelled, and succeeding drops were carefully placed under each half of the cover slip on a modified Neubauer counting chamber. The chamber was then placed on a moistened piece of filter paper in a Petri dish for ten minutes to avoid desiccation and to permit settling of the cells. The number of platelets and erythrocytes in eighty small squares was counted with a high power objective and a 10× ocular. Computation was made in the usual manner. Only a variation of ±200,000 in each count was considered significant.

RESULTS

Extracts of 28 human spleens, and one extract of hepatic and one of lymphoid tissue were prepared. Of these, four spleens, the liver and lymph nodes were obtained as controls. Various responses were obtained. The first two spleens extracted were from patients who had idiopathic hemorrhagic purpura; these spleens were only slightly larger than normal (Table I). The extracts of these two spleens, when injected, produced a dramatic fall of number of thrombocytes of the first rat from a preinjection level of 700,000 in each cubic

*I wish to express my appreciation to Dr. A. E. Osterberg for his assistance in preparation of the tissue extracts.

millimeter of blood to 40,000 in twenty-four hours (Fig. 1). Blood smears, stained with Wright's stain, confirmed the presence of thrombopenia. The thrombopenia was prolonged for seventy-two hours, after which the number of platelets gradually returned to the preinjection level. Moreover, injection of these extracts was followed by a similar response in eleven other normal rats, each of which weighed 200 Gm. All the animals appeared ill at the height of the thrombopenia. At the end of approximately a week these extracts became extremely toxic. Subsequently all animals died within six hours after the injection.[11] Hemorrhage or purpuric spots did not appear, nor could any definite change be noted in the bleeding or coagulation time. The number of erythrocytes did not vary significantly.

TABLE I

CASES IN WHICH THE SPLEEN WAS EXTRACTED

CASE	SEX	CONDITION	WEIGHT OF TISSUE EXTRACTED (GM.)
1	F	Hemorrhagic purpura, idiopathic	115
2	M	Hemorrhagic purpura, idiopathic	110
3	F	Hemorrhagic purpura, idiopathic	80
4	F	Hemorrhagic purpura, idiopathic	90
5	F	Hemorrhagic purpura, idiopathic	55
6	F	Hemorrhagic purpura, idiopathic	64
7	F	Hemorrhagic purpura, idiopathic	120
8	F	Hemorrhagic purpura, idiopathic	100
9	M	Hemorrhagic purpura, idiopathic	117
10	F	Hemorrhagic purpura, idiopathic	104
11	M	Hemorrhagic purpura, idiopathic	95
12	F	Hemorrhagic purpura, idiopathic	78
13	F	Hemorrhagic purpura, idiopathic	195
14	F	Hemolytic icterus	363
15	F	Hemolytic icterus	188
16	F	Hemolytic icterus	160
17	M	Hemolytic icterus	460
18	M	Hemolytic icterus	180
19	M	Banti's splenomegaly	350
20	M	Banti's splenomegaly	320
21	M	Banti's splenomegaly	300
22	M	Banti's splenomegaly	382
23	M	Banti's splenomegaly	282
24	M	Hodgkin's splenomegaly (grade 4 sarcoma type)	590
25	F	Normal spleen	36
26		Normal spleen	140
27		Normal spleen	125
28		Normal spleen	125
29		Normal liver	490
		Normal lymph nodes	25

Three other extracts of spleens obtained from patients who had hemorrhagic purpura produced only a small transitory decrease in the circulating platelets in six hours; one other extract of a spleen from a patient who had hemolytic icterus produced a definite decrease in the number of platelets, which lasted only a few hours. None of these produced the striking thrombopenia of the first two extracts (Fig. 1). These extracts, however, never became toxic, although several animals received injections. The extracts from the remaining tissue did not produce any significant decrease of the number of blood platelets or erythrocytes after injection (Fig. 1). Even injection of larger amounts of the extracts, as high as 20 c.c. failed to reduce the number of platelets. However, all animals ap-

peared apathetic for short periods after the injections, a result suggestive of an indeterminate type of reaction.

COMMENT

Although thrombocytopen could not be extracted consistently from all the spleens of patients who had hemorrhagic purpura, its presence was indicated in some of them. Five extracts of the 13 spleens from patients who had idiopathic hemorrhagic purpura produced a certain amount of thrombolytic activity. Moreover, one of five spleens from patients who had hemolytic icterus apparently contained a thrombocytolytic substance (Table II). The fact that all animals

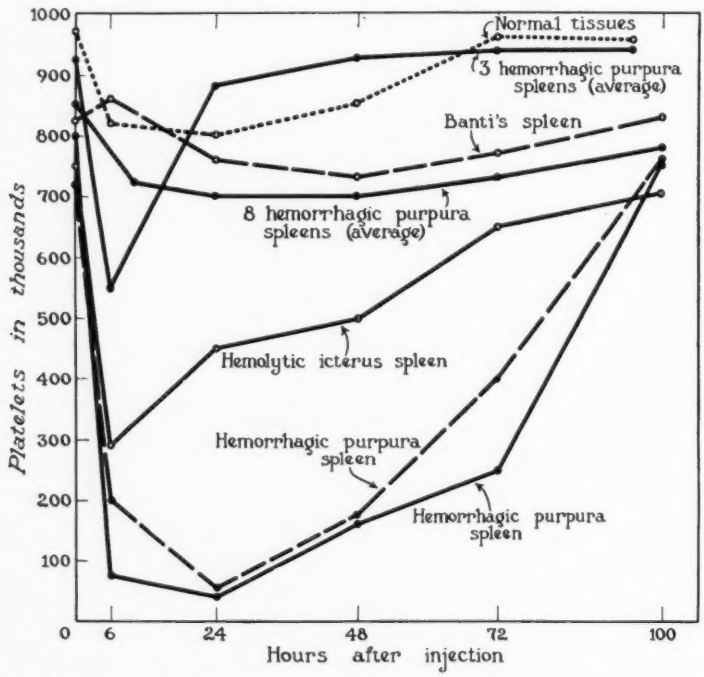


Fig. 1.—Effect of injections of various tissue extracts on the circulating blood platelets of the albino rat.

were apathetic after the injection of the extracts suggests an anaphylactoid response, probably nonprotein in nature since the acetone is believed to remove all the protein. The extracts were impure and the lipoid fraction may have contained substances which produced this effect.

Neither extraction with reagent acetone or with commercial acetone nor distillation of the acetone fraction by various methods seemed to influence the thrombolytic activity of the extracts.

No explanation can be given for the toxic manifestations encountered with the first two extracts used. Dilution of the material and filtration through a Zeiss filter failed to diminish the toxicity. It is not likely that failure of the number of blood platelets to decrease could be attributed to the citrate solution. Platelets counted after dilution with a 2 per cent solution of sodium citrate remained consistently constant prior to injection, and this result suggested that the method was satisfactory. With the Rees-Ecker stain a platelet count that is too high rather than too low is likely to be obtained.

Rats were used instead of rabbits principally because the control platelet counts were more constant and because these animals were more available. Since most of the data in the literature on this problem were derived from experiments on rabbits, some investigators may criticize these results as incomparable with the results of earlier studies. It is doubtful, however, whether the type of laboratory animal used is a factor in influencing the results, since rabbits also were given injections of the extracts in the first few experiments. With rabbits the response of the platelets was not unlike that found among rats.

TABLE II

RESPONSE OF PLATELETS TO INJECTION OF TISSUE EXTRACT

CASES IN WHICH SPLEEN WAS EXTRACTED		DECREASE IN NUMBER OF PLATELETS	
CONDITION	NUMBER	100,000 AND MORE	LESS THAN 100,000
Idiopathic hemorrhagic purpura	13	5	8
Hemolytic icterus	5	1	4
Banti's disease	5		5
Normal spleen	4		4
Normal liver	1		1

SUMMARY

The results of this study neither clearly demonstrate nor definitely deny the existence of a thrombocytolytic substance, "thrombocytopen," in extracts of spleens from patients who had idiopathic hemorrhagic purpura. Because of the inconsistency of the results, the study indicates that a better method of extraction is essential. Twenty-eight spleens, liver tissue, and lymphoid tissue were obtained from patients and the thrombocytolytic substance was extracted.

Thirteen spleens from patients who had hemorrhagic purpura were extracted. Extracts from two produced a considerable drop in the circulating blood platelets; in three they produced a moderate decrease, while the rest gave no response. Five spleens from patients who had hemolytic icterus were extracted; the extract of one spleen apparently lowered the number of platelets. This response was repeated in other animals and was not eliminated by heating the extract.

The decrease of the number of platelets and the lethargic state of the animals after injection suggested the presence of anaphylaxis or a histamine-like reaction, since the symptoms appeared shortly after administration of the extract and disappeared within a few hours.

REFERENCES

1. Colmer, M. L., and Mersheimer, W. L.: Relation of Splenic Extract to Etiology of Essential Thrombopenia, Arch. Surg. **43**: 422, 1941.
2. Hittmair, Anton: Das Blutplättchen im Spiegel des Schrifttums der letzten 10 Jahre, Folia haemat. **59**: 50, 1938.
3. Hobson, F. C. G., and Witts, L. J.: Platelet Reducing Extracts of the Spleen, Brit. M. J. **1**: 50, 1940.
4. Major, R. H., and Weber, C. J.: Is There a Platelet-Reducing Substance in the Spleen of Thrombocytopenic Purpura? J. LAB. & CLIN. MED. **25**: 10, 1939.
5. Otenasek, Frank, and Lee, F. C.: Further Observations on Thrombocytopen, J. LAB. & CLIN. MED. **26**: 1266, 1941.
6. Ottenberg, Reuben, and Rosenthal, Nathan: A New and Simple Method for Counting Blood Platelets, J. A. M. A. **69**: 999, 1917.
7. Paul, J. T.: The Effect of Splenic Extracts From Cases of Essential Thrombocytopenic Purpura on Platelets and Hematopoietic Organs of Rabbits (Abstr.), Proc. Central Soc. Clin. Research **14**: 55, 1941.

8. Peirce, E. G., and Pemberton, Ralph: The Red Cell Count in Arthritis, Arch. Int. Med. **39**: 421, 1927.
9. Pemberton, Ralph: The Etiology and Pathology of Arthritis, J. A. M. A. **87**: 1253, 1926.
10. Pohle, F. J., and Meyer, O. O.: Inability to Demonstrate a Platelet Reducing Substance in an Acetone Extract of the Spleen From Patients With Idiopathic Thrombocytopenic Purpura, J. Clin. Investigation **18**: 537, 1939.
11. Rees, H. M., and Ecker, E. E.: An Improved Method for Counting Blood Platelets, J. A. M. A. **80**: 621, 1923.
12. Rose, Hugh, Jr., and Boyer, L. B.: Thrombocytopen: A Confirmatory Report, J. Clin. Investigation **20**: 81, 1941.
13. Tocantins, L. M.: No Platelet-Destroying Action in Extracts of the Spleen and Urine of Patients With Chronic Thrombopenic Purpura, Proc. Soc. Exper. Biol. & Med. **42**: 485, 1939.
14. Torrioli, M., and Belleli, D.: Studi sulla biologia dei megacariociti sopravviventi in vitro; azione di estratti di milza a concentrazioni scalari, Il Policlinico (sez. med.) **42**: 214, 1935.
15. Torrioli, M., and Galeazzi, M.: Studi sulla biologia dei megacariociti sopravviventi in vitro; azione di estratti di muscolo e di fegato, Il Policlinico (sez. med.) **41**: 647, 1934.
16. Torrioli, M., and Puddu, V.: Studi sulla biologia dei megacariociti sopravviventi in vitro; azione del sangue venose splenico, Il Policlinico (sez. med.) **42**: 129, 1935.
17. Torrioli, M., and Puddu, V.: Studi sulla biologia dei megacariociti sopravviventi in vitro, Il Policlinico (sez. med.) **41**: 245, 1934.
18. Torrioli, M., and Pusic, G.: Azione piastrinopenica elettiva di dosi massive di estratto splenico, Boll. Soc. ital. biol. sper. **9**: 662, 1934.
19. Torrioli, Mario, and Puddu, Vittorio: Recent Studies on Pathogenesis of Werlhof's Disease, J. A. M. A. **111**: 1455, 1938.
20. Troland, C. E., and Lee, F. C.: Thrombocytopen: A Substance in the Extract From the Spleen of Patients With Idiopathic Thrombocytopenic Purpura That Reduces the Number of Blood Platelets, J. A. M. A. **111**: 221, 1938.
21. Troland, C. E., and Lee, F. C.: A Preliminary Report on a Platelet-Reducing Substance in the Spleen of Thrombocytopenic Purpura, Bull. Johns Hopkins Hosp. **62**: 85, 1938.

A PRELIMINARY REPORT ON THE TREATMENT OF BACILLARY DYSENTERY WITH SUCCINYL SULFATHIAZOLE*

EDGAR J. POTH, PH.D., M.D., BEACH M. CHENOWETH, JR., M.D., AND F. LOUIS KNOTTS, M.D., BALTIMORE, MD.

THE introduction of sulfonamide therapy has been the most significant recent advance in the treatment of bacillary dysentery. The dysentery organisms are particularly susceptible to the antibacterial action of several members of this series of drugs. Sulfapyridine[1, 2] and sulfathiazole[3-7] are probably the most effective sulfonamides reported so far. They would be entirely satisfactory if they were less toxic.

Cooper and Keller[8] showed that a single dose of 2 mg. of sulfathiazole given orally at the time of intraperitoneal injection of a suspension of 1,000 minimal lethal doses of *Shigella paradysenteria* Flexner protected mice against the infection. Under similar conditions sulfanilamide was ineffectual.

*From the Department of Surgery and the Department of Pediatrics, the Johns Hopkins University School of Medicine, Baltimore.

This work was supported by a grant from Sharp & Dohme, Philadelphia, Pa.
Received for publication, June 6, 1942.

The successful use of various sulfonamides would indicate that many of these compounds are effective against bacillary dysentery when they are absorbed slowly enough from the gastrointestinal tract to allow the concentration of the drug to reach bacteriostatic levels in the bowel. An important property of an active compound ideally suited to the treatment of bacillary dysentery requires, therefore, that the drug be sparingly absorbed from the bowel. This property will keep the drug where it can be directly active against the bacteria and will minimize general toxicity.

TOXICITY OF SULFAPYRIDINE, SULFATHIAZOLE, AND SULFANILYLGUANIDINE

A review of the literature on the use of sulfapyridine, sulfathiazole, and sulfanilylguanidine shows that these compounds cause toxic reactions when used to treat bacillary dysentery.

Sulfapyridine is the most toxic. Ravenel and Smith[2] state, "In a few patients sulfapyridine produced marked restlessness; this was controlled with phenobarbital."

Welch[9] found more severe manifestatiosn of toxicity: "Sulfapyridine administration is followed by symptoms of nausea and vomiting which occur to varying degrees in 30 to 40 per cent of children under treatment. Too frequently the vomiting makes the therapy disagreeable and too often is so severe that it necessitates discontinuing the drug."

The toxic manifestations due to sulfathiazole are less severe. One patient we treated developed a rash which necessitated discontinuance of sulfathiazole. A more serious sequence was reported by Taylor.[4] An infant, 4 months of age, received 0.9 Gm. of sulfathiazole in the presence of a slight jaundice. The jaundice increased and the drug was discontinued. The child died twenty-three days later of the bacillary dysentery. Although the jaundice might have been due to multiple transfusions of blood, it was for fear of a drug reaction that sulfathiazole was withheld.

Of the three drugs used most widely in the treatment of bacillary dysentery, sulfanilylguanidine appears least toxic. Lyon[10] observed mild toxic manifestations, consisting of (1) disturbances of sensorium, (2) conjunctivitis, and (3) "discoloration" of the skin. These evidences of mild toxicity occurred in four of the 23 cases treated.

SUCCINYL SULFATHIAZOLE AS A CHEMOTHERAPEUTIC AGENT LOCALLY ACTIVE IN THE BOWEL

The experimental and clinical foundation for the use of succinyl sulfathiazole in the treatment of bacillary dysentery was laid by Poth, Knotts, Lee, and Inui[11-13] These authors demonstrated that this compound has much greater local activity against the coliform organisms in the bowel than sufathiazole and sulfanilylguanidine. The physical character of feces is profoundly altered by the action of succinyl sulfathiazole. The feces become practically odorless.

Furthermore, it has been shown that the toxicity of this drug is unusually low. Approximately 5 per cent of the ingested compound is excreted by the kidneys when diarrhea is absent. In the presence of diarrhea an even smaller proportion of the therapeutic dose is recovered from the urine.

TABLE I

RESULTS OF TREATING BACILLARY DYSENTERY IN CHILDREN WITH SUCCINYL SULFATHIAZOLE

NAME	WT. IN KG.	AGE	DURATION DIARRHEA BEFORE TREATMENT	ORGANISM	MAXIMUM TEMPERATURE	NO. OF HOUES TREATMENT REQUIRED TO LOWER TEMPERATURE TO 37° C.	NO. STOOLS DAILY BEFORE TREATMENT	NO. STOOLS ON DAYS OF TREATMENT					DOSAGE GM./KG./DAY*	NO. DAYS TREATED
								1	2	3	4	5		
MN	15.0	3 yr.	8 hr.	Sonne	41° C.	12	Frequent	7	1	0	0	1	0.20 (a)	2½
CL	11.0	2 yr.	12 hr.	Flexner	40° C.	24	Frequent	5	2	1	0	1	0.25 (a)	2
KS	22.4	8 yr.	12 hr.	Flexner	40° C.	18	8	6	0	0	1	0	0.20	5
DT	10.4	3 yr.	24 hr.	Flexner	39.8° C.	12	10-12	6	2	1	3	0	0.3 (b)	4
TY	12.4	3 yr.	24 hr.	Flexner	40° C.	36	8	4	0	2	3	2	0.25 (b)	8
GY	13.0	2 yr.	24 hr.	Sonne	40° C.	12	Many	2	0	1	0	2	0.25	5
JE	11.5	2 yr.	5 days	Flexner	38.5° C.	12	10-15	4	0	3	2	0	0.25 (a)	5
BL	8.2	10 mo.	9 days	Flexner	39.6° C.	24	Many	5	3	1	4	4	0.5 (c)	4½
RR	8.9	14 mo.	13 days	Sonne	38.8° C.	16	8-10	3	0	1	2	1	0.5 (c)	3
MY	3.0	8 wk.	14 days	Flexner	40° C.	24	12	4	6	2	1	3	1.0	6

*All daily dosages are divided into six equal portions and administered at four-hour intervals.
(a)—1 Gm. as an initial dose.
(b)—1.5 Gm. as an initial dose.
(c)—0.75 Gm. as an initial dose.

TREATMENT OF BACILLARY DYSENTERY WITH SUCCINYL SULFATHIAZLE*

The usual general symptomatic treatment was given all patients. This regimen included the administration of adequate parenteral fluids and electrolytes to combat dehydration when the need for this was indicated. No patients received transfusions of whole blood or plasma for the treatment of the dysentery. One patient did receive a single transfusion of whole blood because of a long-standing nutritional anemia. The diagnosis of bacillary dysentery was confirmed bacteriologically in all cases included in this study.

The drug was given by mouth, and the dosage varied from 0.25 Gm. to 1.0 Gm. per kilogram daily, divided into six equal portions. The length of treatment varied from two to seventeen days. As indicated in Tables I and II, there were no failures and no deaths. The patients ranged in age from 8 weeks to 83 years.

TABLE II

BACILLARY DYSENTERY IN ADULTS TREATED WITH SUCCINYL SULFATHIAZOLE

NAME	AGE (YR.)	DURATION OF DIARRHEA BEFORE TREATMENT STARTED	ORGANISM	NO. STOOLS BEFORE TREATMENT	STOOLS DAILY WITH TREATMENT					DOSAGE GM./KG./DAY	NO. DAYS TREATED
					1	2	3	4	5		
ST	21	3 days	Shiga	9	3	2	3	1	1	0.25 (a)	4
RS	22	4 days	Sonne	12	4	3	2	2	2	0.25	4
RS	19	4 days	Flexner	15	9	5	3	1	1	*b*	4
WW	29	7 days	Flexner	15-18	14	9	6	2	1	*b*	4
MM	23	2 days	Flexner	20-25	14	9	3	1	1	*b*	4
HS	23	6 days	Flexner	20-25	14	4	2	1	1	*b*	4
JO	25	1 day	Not typed	12-15	12	3	1	1	1	*b*	4
SS	30	2 days	Flexner	25-30	20	7	8	2	1	*b*	6
DN*	83	32 days	Flexner	10-15	4	1	8	8	4	*b*	17
HY	57	3 mo.	Flexner	Many	4	1	2	1	1	0.25 (a)	14

*This patient was in extremely poor condition, with malnutrition and decubitus ulcers, and showed a rash following sulfathiazole therapy without benefit to dysentery before succinyl sulfathiazole treatment was instituted. Treatment was continued much longer than necessary.

(a)—0.25 Gm. per kilogram as a single initial dose.

b —0.5 Gm. per kilogram daily for two days, then 0.25 Gm. per kilogram daily. Daily dosage is divided into six equal portions and given at four-hour intervals.

The data recorded in Table I indicate that the response of children and infants suffering from bacillary dysentery when treated with succinyl sulfathiazole is prompt whether treatment is begun early or late in the disease. The response is equally good with the smaller doses as with the larger. The temperature returned to normal in twenty-four hours or less in all instances except one. In this case the temperature became normal in thirty-six hours. The results of treating ten adults with bacillary dysentery with succinyl sulfathiazole are summarized in Table II. The response to therapy is again prompt regardless of the duration of the disease.

The number of patients in this series was insufficient to determine accurately the minimum dosage of drug and the shortest period of therapy required for the successful treatment of bacillary dysentery with succinyl sulfathiazole. It is obvious, however, that in most instances unnecessarily large quantities of drug were given for longer periods than required. In fact, the response was better in those patients receiving the smaller doses. Bacteriologic studies showed the *Shigella paradysenteriae* organisms to be especially susceptible to the anti-

*The succinyl sulfathiazole (sulfasuxidine) used in this study was supplied by Sharp & Dohme, Philadelphia, Pa.

bacterial action of succinyl sulfathiazole. The dysentery organisms ordinarily disappeared from stools within forty-eight hours, whereas significant lowering of the coliform bacteria required two or three days of additional therapy.

It is especially significant that the response to succinyl sulfathiazole is immediate, although the disease has been present for as long as three months before treatment is undertaken. This result is in contradistinction to the observations made by Lyon and Marshall regarding the efficacy of sulfanilylguanidine. As stated by Lyon:[14] "The drug appears to be most efficacious when administered during the first 3 to 4 days after onset of fever or diarrhea. Although the percentage of failures may be higher when the drug is not given until after the fifth day of illness, it has been observed to be effective at times even when given later in the disease."

Marshall, Bratton, Edwards, and Walker[15] say: "Ten children were treated late in the disease, the institution of chemotherapy varying from the 4th to the 14th day of the disease. In this group the results were not uniform. Some cases showed the same striking improvement that occurred in the children treated early, but the majority ran a course uninfluenced by the administration of sulfanilylguanidine."

It appears that sulfanilylguanidine is most effective against bacillary dysentery if the treatment is started early. It may fail to influence the course of the disease if treatment is delayed.

DISCUSSION

While the results of treating bacillary dysentery with the sulfonamides are so good in the United States, it must be realized that this outcome is partly due to the excellent state of nutrition usually encountered here. In areas where states of poor nutrition exist, especially among children, mere bacteriostasis does not give such brilliant results, because the diarrheas of the specific bacillary dysenteries are complicated by malnutrition. Under these circumstances attention to general care, fluid, and electrolyte replacement, and nutrition must be held of equal importance with specific drug therapy.

Under ordinary conditions of our national civilization, sanitary safeguards and food protection are developed to a high degree and bacillary dysentery among adults is not a serious problem. In the armed forces, however, men in strange climates and frequently in unsanitary surroundings are unduly exposed, and infection of the adult becomes a problem of major importance. Because of the apparent great efficacy of succinyl sulfathiazole in bacillary dysentery and the absence of toxic symptoms due to the administration of this drug, it would be highly desirable to consider the use of succinyl sulfathiazole as a prophylactic agent. A small quantity of drug given in the daily ration of men in areas of heavy exposure might offer protection. Such a protective dose might be surprisingly small; and in the absence of any effect on the sensorium, as determined by reaction time, and so on, the drug could be administered to great advantage.

The doses given in the cases of bacillary dysentery reported here may well be much larger than is required. No toxicity to the drug has been observed in any of these patients, and there were no instances where the drug failed to ar-

rest the disease in a very short time. Since the minimum effective therapeutic dose of succinyl sulfathiazole has obviously not been established, a satisfactory prophylactic dose might be surprisingly small.

Because sulfathiazole is highly effective in bacillary dysentery, because the activity of succinyl sulfathiazole is probably due to the degradation product, nascent sulfathiazole, and because succinyl sulfathiazole can be maintained in high concentration in the bowel with only slight absorption and without the development of toxic manifestations, it is not surprising that succinyl sulfathiazole in tolerated therapeutic doses should have a pronounced antibacterial activity against the organisms of bacillary dysentery. In all fairness it should be stated that during the past summer bacillary dysentery was not prevalent, and the disease might have been relatively mild. This communication should, therefore, be regarded as a preliminary report. Kirby and Rantz[16] have, however, succeeded in curing bacillary dysentery carriers with succinyl sulfathiazole.

SUMMARY

The treatment of bacillary dysentery with a new chemotherapeutic agent, succinyl sulfathiazole, is presented. The drug is equally effective in both the acute and the more chronic forms of the disease. All cases of bacillary dysentery treated responded promptly. The administration of the drug caused no untoward toxic manifestations.

We wish to thank Dr. Samuel Revell, of the University of Maryland School of Medicine, and Lt. David W. Exley, of Camp Jackson, South Carolina, for the privilege of including in this report cases of bacillary dysentery treated under their direction.

REFERENCES

1. Reutler, R., and Marberg, K.: Note on the Treatment of Acute Bacillary Dysentery With Sulfapyridine, Brit. M. J. p. 277, Feb. 22, 1941.
2. Bell, G. J.: Treatment of Bacillary Dysentery With M & B 693, Lancet **241**: 101, 1941.
3. Anderson, E. V.: The Chemotherapy of Infectious Diarrhea With Sulfathiazole, J. Pediat. **18**: 732, 1941.
4. Taylor, G.: Sulfathiazole Therapy of Infantile Diarrhea, J. Pediat. **18**: 469, 1941.
5. Lawrence, C. A.: Bacteriostatic Effects of Sulfanilamide, Pyridine, Thiazole Derivatives Upon Colon-Typhoid-Dysentery Group, Proc. Soc. Exper. Biol. & Med. **44**: 162, 1940.
6. Ravenel, S. F., and Smith, D. L., Sr.: The Chemotherapy of Bacillary Dysentery, South. M. J. **34**: 509, 1941.
7. Cooper, M. L., Zucker, R. L., and Wagoner, S.: The Treatment of Infantile Dysentery With Sulfathiazole, J. A. M. A. **117**: 1520, 1941.
8. Cooper, M. L., and Keller, H. M.: Sulfonamide Drugs in the Treatment of Experimental Dysentery Infection in Mice, J. Pediat. **18**: 460, 1941.
9. Welch, S. H.: In Discussion, South. M. J. **34**: 510, 1941.
10. Lyon, G. M.: Sulfanilylguanidine: Treatment of Acute Bacillary Dysentery, U. S. Nav. M. Bull. **39**: 286, 1941.
11. Poth, E. J., and Knotts, F. L.: Succinyl Sulfathiazole. A New Bacteriostatic Agent Locally Active in the Gastrointestinal Tract, Proc. Soc. Exper. Biol. & Med. **48**: 129, 1941.
12. Poth, E. J., Knotts, F. L., Lee, J. T., and Inui, F.: Bacteriostatic Properties of Sulfanilamide and Some of Its Derivatives. I. Succinylsulfathiazole, a New Chemotherapeutic Agent Locally Active in the Gastrointestinal Tract, Arch. Surg. **44**: 187, 1942.
13. Poth, E. J., and Knotts, F. L.: Clinical Use of Succinyl-sulfathiazole, Arch. Surg. **44**: 208, 1942.
14. Loc. cit., page 293.
15. Marshall, E. K., Jr., Bratton, A. C., Edwards, L. B., and Walker, Ethel: Sulfanilylguanidine in the Treatment of Acute Bacillary Dysentery in Children, Bull. Johns Hopkins Hosp. **68**: 101, 1941.
16. Kirby, W. M. M., and Rantz, L. A.: The Treatment of Typhoid and Dysentery Carriers With Succinyl Sulfathiazole, J. A. M. A. **119**: 615, 1942.

A NEW HYPOTHESIS OF THE PRODUCTION OF THE T WAVE IN THE ELECTROCARDIOGRAM BASED ON ELECTROKINETIC PHENOMENA*

J. Roscoe Miller, M.S., M.D., F.A.C.P., and Roy F. Dent, B.S., M.D.
Chicago, Ill.

THE purpose of this paper is to describe a possible mechanism for the production of the T-wave component of the electrocardiogram and the experimental procedure followed in its preliminary study. This study was begun with the hope that a more logical and concrete explanation of the electrobiologic phenomena responsible for this phase of the cardiogram could be found than those explanations now current.

In general, it is accepted that the T wave represents the disappearance of the electric disturbance responsible for the QRS deflection, which phenomenon has been variously termed repolarization, return to the resting state, end deflection of the QRS, etc. The exact manner in which this occurs, however, is disputed. The earlier hypothesis states that the character of the T wave is determined by the vectorial summation of the last portions of two out-of-phase monophasic waves.[1-5] More recently, with the better understanding of the nature of the QRS complex and its relation to myocardial excitation, there has developed the concept that the T wave represents a simple retreat of the wave of excitation, i.e., doublets of retreat.[6]

Without entering into a detailed examination of the two exact mechanisms, either explanation, if correct, should answer questions directed at the broader concept that the T wave is a disappearance phenomenon of an electric disturbance which in its invasion of the myocardium inscribes the QRS complex. One question which immediately rises is why the disappearance of the wave of excitation normally should involve a larger quantity of electricity than that involved in its entrance or invasion. This disparity can be demonstrated readily by comparing the sum of the areas under the QRS and T waves in the normal electrocardiogram taken from enough points over the body surface to encompass the heart completely.[9]† Other questions dealing with specific changes in the QRS without corresponding changes in the T wave, and vice versa, may be asked, and many will be unanswered. For instance, it is a common observation that the T waves increase in magnitude in hyperthyroidism without a corresponding increase in the QRS. If they were an expression of different phases of the same phenomenon, this hardly would seem likely.

*From the Department of Medicine, Northwestern University Medical School, Chicago.
Received for publication, June 15, 1942.

†The electrocardiograph machine is essentially a recording voltmeter which draws the minimum current from the electrical source. Thus the electrocardiogram is a potential-time tracing and the areas under the tracing are a measure of the quantities of electricity involved in each portion of the electrical cardiac cycle.

Another reason for this investigation deals not with the inadequacies of the current explanations but with the inherent electrodynamic characteristics of the T wave itself. A close study of the normal ventricular QRS-T complex shows that the QRS component and the T component are entirely different in form, duration of existence, and rate of change. The QRS tracing has a steep front and an equally steep decline, while the T-wave has a smoothly changing front, a more gradual rate of change, and persists for a much longer time. If the T-wave potential were dependent on cardiac muscular contraction, it would seem that the shape of the tracing could be correlated with a possible mechanism of development; that is to say, the shape would more nearly reflect its manner of production. Further, the greater quantity of electricity involved in the T wave could be explained, for in contracting the heart does work and is, therefore, potentially capable of producing electricity. Obviously, this idea would be untenable at once, were the T wave to be inscribed at any period in the cardiac cycle other than that of cardiac systole, or even begin before or persist after systole. This, however, is not the case, as can be shown by simultaneous intraventricular pressure and electrocardiographic recordings.

The association of the T-wave potential with myocardial activity is not new. Several interesting references to this possibility have appeared in the literature.[7, 8] One reference is to the specific idea to be outlined in the following paragraphs.[9]

In considering the conditions obtaining in the myocardium during systole which could convert mechanical energy into electrical, it is reasonable that those which are involved in a displacement phenomenon are the most likely subjects of investigation. On this hypothesis, then, the intramural displacement of fluids, both intercellular and capillary, suggests itself as a possible source by reason of the electrokinetic phenomen of streaming potentials.[10] The question is, will there by a sufficient pressure gradient along the intramural paths open to the blood, tissue fluid, and lymph to produce an appreciable electrokinetic effect?

From the equation for streaming potentials,

$$H = 8.48 \times 10^{-8} \frac{\zeta \ D \ P}{n \ K_S}$$

where H = Stream potential in millivolts
ζ = Potential of the double layer
n = Coefficient of viscosity
D = Dielectric constant
P = Pressure in cm. Hg
K_S = Specific conductivity in mhos

it is seen that for a given capillary system, the potential is directly proportional to the pressure, the dielectric constant, and the zeta potential; and inversely proportional to the viscosity and the conductivity. A simple substitution of the constants for whole blood at body temperature and an assumed value of 50 millivolts for the potential of the double layer will show, however, that the pressure will have to be high if the potential is to be of the order of magnitude necessary to produce the T wave. Calculations will readily show that ventricular

intramural stress can attain values of 40 cm. Hg throughout most of the wall and values of 60 cm. Hg at the base where the muscle fibers taper down to insert in the valve rings and intervalvular fibrosa.

As a preliminary test of the foregoing reasoning, dead hearts were perfused through the left coronary artery with various fluids (5 per cent glucose, 0.2 to 0.9 per cent sodium chloride, whole heparinized blood), and the potential across the anterior wall of the left ventricle was measured at various intramural pressures. At pressures between 20 and 40 cm. Hg significant potentials were developed, and the variations were in agreement with those predicted from the equation, namely, that the low conducting solutions produced the largest potential and the more conducting solutions the smaller potential, and that the potential was directly related to pressure. The potential developed with blood, which is the one of interest, was 0.5 millivolts at 40 cm. pressure. It should be noted here that the potentials developed varied considerably with the individual hearts and appeared to depend on the time dead, since higher values were obtained with the fresher material. These experiments, therefore, are not quantitative but qualitative, and the results are of value only in that they demonstrate that the internal architecture of the ventricular wall affords a capillary system which, when there is streamed through it a fluid with the comparatively high electrolyte concentration of whole blood, is capable of producing significant streaming potentials.

The question of the exact manner in which the blood and extracellular fluid may be displaced or squeezed about during ventricular systole to produce streaming potentials, cannot be answered dogmatically. The most logical sequence would seem to be a progressive "wringing out" of the myocardium as contraction progresses to a maximum. That blood, at least, is "wrung" out during systole is shown by the coronary (venous) outflow which occurs at this time in the cardiac cycle.[11, 12] For any given segment of myocardium, the sign of the developed potential would be negative to positive when progressing in the direction of the intermuscular fluid displacement.

From the foregoing it can be postulated that regardless of the manner in which an intramyocardial pressure gradient is produced, whether by the actively contracting heart or by a force applied externally, just so long as it exists flow potentials should be developed. Thus, external energy applied to a heart rendered unresponsive to electrical and mechanical stimuli should produce a potential the characteristics of which are similar to those of the normal T wave as regards magnitude and rate of change. With this idea in mind, then, the following experiments were performed.

Series I.—Method. Dogs anesthetized with nembutal were given 40 c.c. of a 20 per cent solution of magnesium sulfate intravenously in order to decrease the irritability of the myocardium.[13] The thoracic cavity was then opened, care being taken to keep the field dry. The animal was connected through electrodes to the right foreleg and left hind leg to a string galvanometer electrocardiograph. The heart was then stimulated by means of a probe, and records were made of the resulting ectopic impulses. When stimulation ceased to produce an impulse, the heart was grasped in the hand which had been insulated by means of a rubber glove, and roughly bumped about the chest cavity to determine the amount of potential, if any, developed by such a procedure. With the electrocardiograph

recording the heart was then forcibly constricted and suddenly released, or pressure was maintained for a period of several seconds.

Results. Cardiac stimulation produced ectopic impulses for a period varying up to twenty minutes after the chest cavity was opened. As will be seen from Fig. 1*A* a period was finally reached when stimulation produced an impulse without demonstrable contraction or T wave. Fig. 1*B* shows the result of movement of the heart and mediastinal structures. As will be seen, no significant potential was developed despite the fact that the contents of the chest were moved about vigorously, and the string was loosened to exaggerate the effect of any movement. Fig. 1*C* shows the result of constriction of the heart before irritability had entirely disappeared. Here it will be seen that there was a sudden production of a potential which gradually returned toward zero.

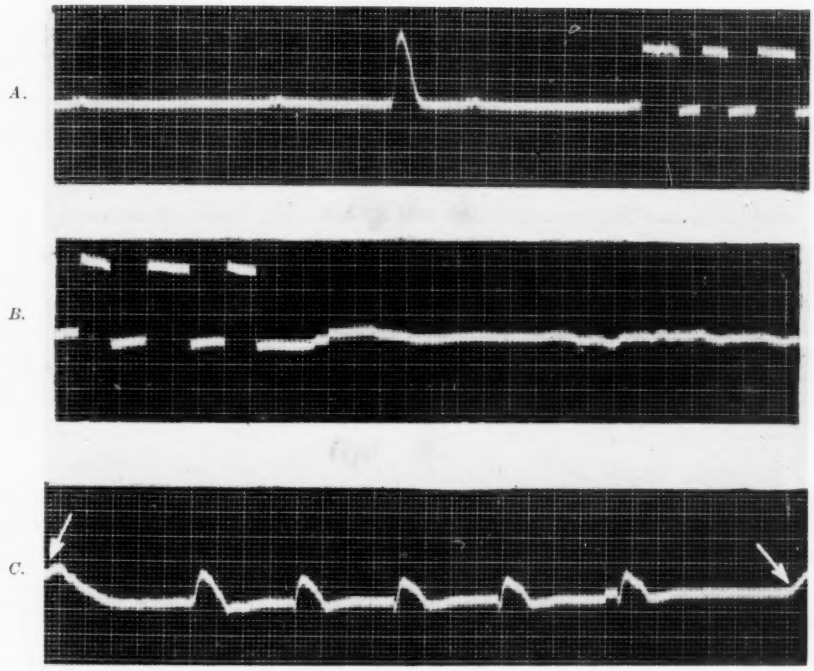


Fig. 1.—*A*, Stimulation of myocardium producing an impulse without demonstrable contraction or T wave. *B*, Vigorous movement of the heart and mediastinal structures. *C*, Arrows indicate application and relaxation of pressure. Note ectopic impulses occurring during this period.

At the same time several ectopic impulses arose from the yet irritable muscle, thus demonstrating that the potential that developed by squeezing the heart was independent of spontaneous impulses. Fig. 2*A* shows the result of sudden constriction of the heart with immediate relaxation, while Fig. 2*B* shows constriction with maintenance of pressure and the return of the potential to zero upon relaxation.

After irritability had ceased there was a period of from five to twelve minutes when the above results could be obtained at will. At the end of that time changes had occurred in the tissue which resulted in adventitious voltages due to development of local concentration cells, so that any movement of the thoracic structures would produce unlimited bizarre potentials.

Series II.—Method. These experiments were conducted to confirm previous observations regarding the time relationship of changes in intraventricular pressure to the T wave in the electrocardiogram. This was done by simultaneously recording on the electrocardiogram the pressure in the left ventricle and carotid artery.[14]

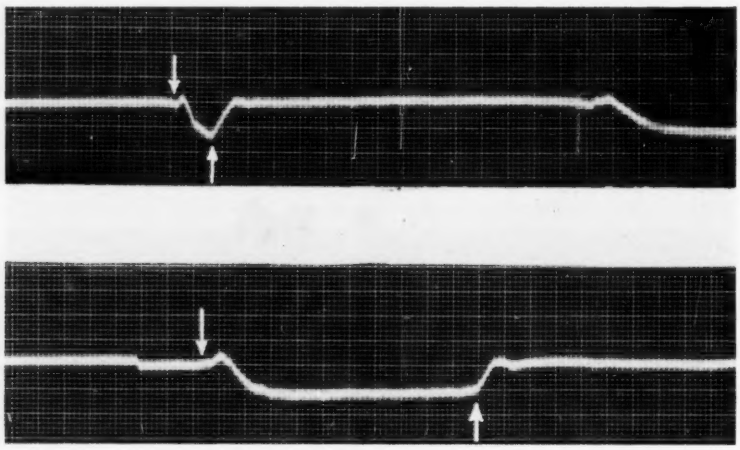


Fig. 2.—*A*, Constriction of heart with immediate relaxation. *B*, Constriction with maintenance of pressure and sudden relaxation.

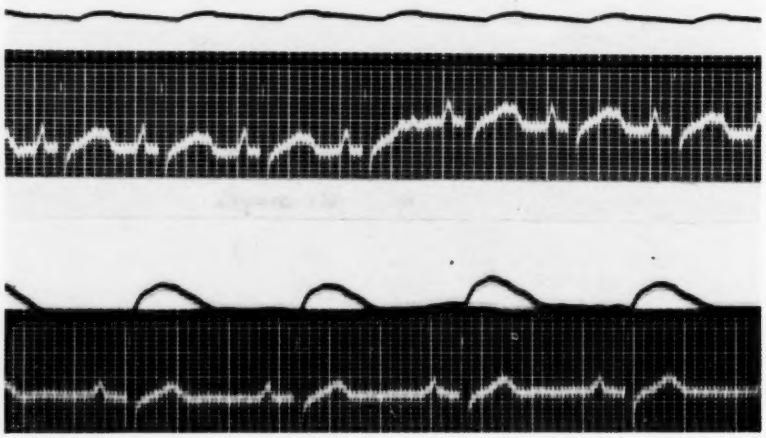


Fig. 3.—*A*, Simultaneous recording of carotid pressure and electrocardiogram. *B*, Left intraventricular pressure and electrocardiogram.

Results. Fig. 3*A* shows the correlation of increases in carotid pressure with the T wave and Fig. 3*B* shows changes in the left intraventricular pressure and the relationship of these changes to the T wave. It will be seen that in each case rise of pressure occurs at the same time as the T wave and that the latter is always confined within the time limits of the former.

SUMMARY AND CONCLUSIONS

The nature of the T wave in the electrocardiogram suggests that it is due to an electrokinetic cause and does not represent retreat electric disturbances.

The structure of the heart with its capillary bed is such that contraction could produce streaming potentials of the order of magnitude of the T wave. Pressure perfusion of the heart showed this to be entirely possible. Constriction of the unresponsive heart likewise produced this phenomenon. In addition it is shown that the T wave corresponds in time to the increase in pressure in the left ventricle. These observations suggest that contraction of the heart with its resultant streaming potentials is responsible for the T wave in the electrocardiogram.

REFERENCES

1. Adrian, E. D.: The Recovery Process of Excitable Tissues, J. Physiol. **55**: 193, 1921.
2. Yoshida, H.: Zur Deutung des Elektrokardiogramms. I. Das Elektrogramm der Ventrikelspitze des Froschherzens, Ztschr. f. Biol. **84**: 51, 1926.
3. Yoshida, H.: Zur Deutung des Elektrokardiogramms. II. Versuche am ganzen, frei aufgehängten Froschventrikel, Ztschr. f. Biol. **84**: 453, 1926.
4. Lewis, Sir Thomas: The Mechanism and Graphic Representation of the Heart Beat, London, 1925, Shaw & Sons, Ltd.
5. Katz, L. N.: The Significance of the T-Wave in the Electrogram and Electrocardiogram, Physiol. Rev. **8**: 447, 1928.
6. Craib, W. H.: The Electrocardiogram, Medical Research Council, Special Report Series, No. 147, London, 1930, H. M. Stationery Office.
7. Samojloff, A.: Weitere Beiträge zur Elektrophysiologie des Herzens, Arch. f. d. ges. Physiol. **135**: 417, 1910.
8. Hill, A.: Analysis of the Normal T-Wave, Lancet **2**: 979, 1939.
9. Krasno, M. R., Eyster, J. A. E., and Maaske, C. A.: The Nature of the T-Wave Potentials in the Tortoise Heart, Am. J. Physiol. **114**: 119, 1935.
10. Abramson, H. A.: Electrokinetic Phenomena, New York, 1934, The Chemical Catalog Co.
11. Anrep, G. V., Cruikshank, E. W. H., Downing, A. C., and Rau, A. S.: The Coronary Circulation in Relation to the Heart Cycle, Heart **14**: 111, 1927.
12. Anrep, G. V., and King, B.: The Significance of Diastolic and Systolic Blood Pressures for Maintenance of Coronary Circulation, J. Physiol. **64**: 341, 1928.
13. Miller, J. Roscoe, and Van Dellen, T. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate, An Experimental Study on Dogs, J. Lab. & Clin. Med. **23**: 914, 1938; **24**: 840, 1939.
14. Hamilton, W. F., Brewer, G., Brotman, I.: Pressure Pulse Contours in the Intact Animal. I. Analytical Description of a New High Frequency Hypodermic Manometer, Am. J. Physiol. **107**: 427, 1934.

CLINICAL CHEMISTRY

THE SIGNIFICANCE OF THE URINARY AMMONIA*

A. P. Briggs, Augusta, Ga.

CONSIDERABLE evidence has accumulated which indicates that it may be necessary to abandon one of our most time-honored concepts: the belief that secretion of ammonia by the kidneys serves in some way to maintain the acid-base balance of body fluids. Indeed, there is reason for suspecting that it has been carried along for a good many years by a combination of inertia and complacency.

The first note of dissension came in 1932 when it was observed that the acidosis of nephritis was not necessarily of the base deficit type, although the capacity for production of ammonia in this state was definitely subnormal. It was pointed out then that all observed facts relating to the secretion of ammonia were consistent with the hypothesis that ammonia production simply operates as a local protective mechanism to prevent excessive tubular acidity

Subsequently a number of special instances were observed in which the activity in production of ammonia was not that to be expected from the conventional theory but more in accord the dissenters' viewpoint.

This evidence will be reviewed briefly in the following sections and certain evidence provided by recent developments in the field of renal physiology will be presented.

CRUCIAL EXPERIMENTS

It should be obvious that the classical concept is based on circumstantial evidence which is perfectly compatible with either view, and that the whole question appears, superficially, to be merely a matter of words. Increased production of ammonia was observed to accompany increased excretion of acid, administered or metabolic. The ammonia was previously thought to be formed from neutral urea by the liver and perhaps by other tissues; secretion of a part of such acid in the form of ammonium salt was assumed to spare fixed alkali of the blood plasma. This assumption was entirely justified up to the time of the publication of the work of Nash and Benedict,[1] which indicated the kidney to be the organ of ammonia production. But the assumption, which has generally been made since then, that substitution of ammonia is part of the mechanism through which fixed base is reabsorbed from the glomerular fluid, and that it responds to requirements of the body for conservation of fixed base, is entirely gratui-

*From the Departments of Biochemistry and Internal Medicine of the University of Georgia Medical School, Augusta.

Received for publication, August 12, 1942.

tous—it is just as easy to suppose that production of ammonia by the tubules *follows* the reabsorption of fixed base as a result of irritation from rejected acid in the tubular fluid.

Since the acid-base balance of the blood and urine each deviates from the normal in the same direction, in most clinical and experimental states, it is impossible to tell from studies of such states whether fluctuations of ammonia are following changes in the plasma or renal tubules. There are available, however, a few studies in which the acid-base balance of the urine fluctuates in a direction opposite to that of the plasma. Such studies give crucial evidence.

EXPERIMENTS WITH SODIUM SALTS OF LOW THRESHOLD ACIDS

Hendrix and Sanders,[2] extending an experiment of Cushny,[3] observed that an acid urine may be formed following the administration of dibasic sodium phosphate or sodium hippurate, because the low threshold acids are not so actively reabsorbed by the renal tubules as is sodium. They also observed that production of ammonia was increased under these conditions in spite of the fact that the blood was "undoubtedly more alkaline." In my own studies with the sodium salt of another low threshold acid, sulfuric, similar changes were observed; the results were clear-cut following subcutaneous injection in the dog.[4]

The fluctuations of ammonia in these experiments, it should be observed, were parallel to the acid-base changes in the urine and were opposite to those of the plasma. They show clearly that production of urinary ammonia is not determined by the needs of the body for conservation of fixed base, for in each case the body was burdened with an excess of sodium. They strongly suggest that it is the acidity of the fluid flowing through the tubules which determines the level of ammonia production.

EXPERIMENTS WITH POTASSIUM CHLORIDE

Potassium chloride is a neutral salt which makes the urine alkaline for the reason that potassium, a low threshold base, is excreted much more rapidly than chloride. Consequently, the residual chloride which is left within the body causes an acid-base shift in the direction of acidity, similar to that which follows the administration of calcium chloride or hydrochloric acid.

Experiments with potassium chloride, administered orally or subcutaneously, have shown ammonia production to be immediately suppressed.[4] They make it clear that an excess of acid within the body is not an adequate stimulus for ammonia production. Again there is the suggestion that the acidity of the fluid flowing through the tubules is the determining factor.

THE INFLUENCE OF DIURESIS ON WASTE OF FIXED BASE AND ON AMMONIA PRODUCTION

The diuresis caused by drinking distilled water or by intravenous infusion of solutions of neutral substances, such as urea, results in the urinary waste of sodium bicarbonate. Presumably the rapid rate of flow through the tubules handicaps the mechanism of reabsorption of this vital plasma alkali. In the diuresis studies of Hendrix and Calvin[5] the plasma bicarbonate was lowered by as much as 15 per cent. But the ammonia mechanism fails to respond to the

base-deficit type of acidosis so produced,[5-7] as it certainly should if it followed needs of the body for conservation of fixed base. This failure is in perfect harmony with the view which relates stimulation of ammonia production to increased tubular acidity. Such a diuresis does not produce an acid urine.

THE CONSERVATION OF FIXED BASE IN NEPHRITIS

There has been some confusion and controversy concerning the influence of nephritis on the renal mechanism for conserving fixed base. It is true that the capacity for production of ammonia is impaired with the destruction of many nephrons, and in such cases there is, occasionally, some evidence of base waste or depletion. The confusion is chiefly due to the obligation usually felt for incriminating the deficient ammonia mechanism as a causative factor—almost always it is possible to point to some other factor which might just as well be responsible.

Low levels of plasma fixed base are frequently encountered with the onset of uremia in nephritis, but vomiting is also frequent at this stage and is a very potent cause for such low levels. In a selected group of patients[8] who had advanced nephritis but who were not vomiting, the plasma fixed base values fell between 145 and 152 milliequivalents per liter; normal values with the same technique fell between 148 and 155. This slight difference, while hardly significant, might well be a diuresis effect. These patients had a polyuria and relatively few surviving nephrons. Consequently the rapid rate of tubular flow should have offered some handicap to tubular reabsorption.

If depressed levels of plasma fixed base result from the excretion of acid which would normally be carried off by ammonia, then prolonged administration of acid should have a very pronounced effect in lowering these levels in nephritis. Such, however, is not the case. In the studies of Linder,[9] Gamble, Blackfan, and Hamilton,[10] as well as that of my own,[8] there was no significant change in the level of plasma fixed base, in either normal or nephritic subjects as a result of heavy dosage of acid. This is one of the most important observations relating to the question of base waste in nephritis.

In certain instances an excessive amount of fixed base has been found to be excreted into the urine of nephritic patients as a result of acid administration. The urinary excretion of the various acids and bases was determined in each of the above-mentioned studies of acid ingestion. Gamble and co-workers observed that excretion of extra fixed base by their nephritic patient was excessive in comparison with their normal controls. However, this was a patient with an edematous type of nephritis, and so the significance is uncertain—delivery of edema fluid from any cause should result in the elimination of extra fixed base. Linder's study gave complete results on two nephritic subjects without edema. One excreted an excessive amount of fixed base, while the other did not. In my own study one of three nephritic subjects excreted an excessive quantity of fixed base, but this was accompanied by a conspicuous diuresis—twenty-four-hour urine volumes greater than 3,000 c.c.

The evidence for impaired conservation of fixed base in nephritis is certainly inconclusive, and the incrimination of the defective ammonia mechanism for such evidence as is available seems quite unnecessary.

THE EVIDENCE OF RECENT DEVELOPMENTS IN RENAL PHYSIOLOGY

The results of much critical study[11] tend to support the essentials of the "modern theory of urine formation," as elaborated by Cushny. The glomerular fluid seems to be a true filtrate which is formed, in an adult, at the rate of about 120 c.c. per minute. It also appears that the resultant fluid formed by reabsorption in the various compartments of the tubule system must be of fairly constant composition, especially so in regard to the constituents of the basic elements; otherwise the levels of these constituents in the plasma would not be maintained within their observed narrow ranges. The tubules do, however, secrete a few substances. Perhaps the most important of these is ammonia, the secretion of which in the amphibian tubule has lately been reported by Walker.[12]

QUANTITATIVE RELATIONS BETWEEN SECRETION OF AMMONIA AND REABSORPTION OF FIXED BASE

The notion that secretion of ammonia is necessary for reabsorption of fixed base probably rests on the failure to bear in mind the quantitative relations of the two processes. With glomerular filtration at the rate of approximately 120 c.c. per minute, and with a fixed base concentration of about 150 meq. per liter, something like 1,080 meq. per hour of fixed base is filtered. Consequently, with the urinary excretion of fixed base at levels varying from 2 to 20 meq. per hour, the magnitude of fixed base reabsorption is seen to be at the enormous level of more than 1,000 meq. per hour. Under ordinary conditions the output of ammonia amounts to something less than 3 meq. per hour. It is, therefore, perfectly clear, when the magnitudes of these two processes are contrasted, that reabsorption of fixed base does not depend on substitution of ammonia. The highest levels of ammonia production, such as those observed in diabetic acidosis, are of the order of 25 meq. per hour, and so even these are hopelessly inadequate in accounting for fixed base reabsorption. The comparison is somewhat improved by making an additional assumption, which would bestow on the renal epithelium the capacity for differentiating between the base opposed by bicarbonate and the base opposed by chloride and other strong acid; ammonia to be substituted only for base opposed by bicarbonate. But this fraction of the total fixed base amounts to nearly 200 meq. per hour, which is again far greater than the maximum observed levels of ammonia production.

WORK ON THE ISOLATED NEPHRON OF AMPHIBIA

This work has been done on material aspirated directly from various levels of the tubule. In the proximal tubule there is no secretion of ammonia,[12] but glucose is reabsorbed and presumably along with it a considerable amount of water and salt.[13] The fixed base of this salt is therefore reabsorbed without substitution of ammonia. Actually ammonia was not found to appear before the distal half of the distal convoluted tubule, but chloride was actively reabsorbed by the first part of the distal tubule, and without change in pH,[14] so it must have been accompanied by an equivalent quantity of fixed base. Reabsorption of additional fixed base is thus accomplished without substitution of ammonia.

Acidification of the urine was observed to occur in a narrow zone near the middle of the distal tubule. The process of acidification was thought to depend on reabsorption of base bicarbonate. Since it was at this zone of acidification that secretion of ammonia was found to be initiated by Walker,[12] it is of great interest to speculate on the relation of the two processes. It is evident that ammonia is not substituted for all the base bicarbonate reabsorbed in this zone, for if so there would be no acidification. The manner in which ammonia is usually thought to operate is by interaction with salts of the glomerular fluid according to the reversible equilibrium:

$$NH_4HCO_3 + BAc \rightleftharpoons NH_4Ac + BHCO_3$$

According to this scheme, base bicarbonate would be reabsorbed and the acid radical would be carried off into the urine as ammonium salt.[11] But ammonia has been observed to be secreted only in the presence of acid.[12] Consequently, such a reversible equilibrium could never get anywhere in either direction because of the vigorous interaction of ammonia with acid, already liberated according to the scheme:

$$NH_4HCO_3 + HAc \rightarrow NH_4Ac + H_2CO_3$$

This reaction would very rapidly be carried to completion because of the slightly dissociated character of H_2CO_3.

Since ammonia reacts with acid which **results** from reabsorption of base bicarbonate, it might be said that the arrival of ammonia, unfortunately, is too late for the rescue. The simultaneous appearance of ammonia and acid formation is, to say the least, suggestive that secretion of ammonia operates as a local protective mechanism to prevent excessive acidity.

STUDIES OF THE MAMMALIAN NEPHRON

Preliminary reports from two separate laboratories[15, 16] each indicate that reabsorption of bicarbonate, that is acid production, may occur in the proximal tubule of the mammalian nephron. If this is true, it means there can only be a limited field of action for interchange of ions between ammonium bicarbonate and salts of the glomerular filtrate—even if secretion of ammonia should be found to occur at a level above the zone of acidification. If, as in Amphibia, secretion of ammonia is initiated at the zone of acidification, then the interpretation will be that indicated above; the classical concept relating secretion of ammonia to conservation of fixed base will have to be radically revised.

SUMMARY AND CONCLUSIONS

Crucial experiments show that ammonia production can, at will, be made to fluctuate in a direction opposite to that of any given acid-base change in the body. In all such experiments ammonia production fluctuates in the same direction as the acid-base change of the urine.

With advanced nephritis, in spite of curtailed production of ammonia, there is no clear-cut evidence of impaired conservation of fixed base. The evidence which is available does not necessarily incriminate the ammonia mechanism. Other factors, such as vomiting or diuresis, are usually present in instances of defective base conservation.

Examination of fluid taken from various levels of the amphibian nephron reveals that secretion of ammonia *follows* reabsorption of fixed base; and that ammonia is only secreted in the presence of acid, which precludes an interchange of ions in the reversible equilibrium usually assumed to be involved in the substitution of ammonia for fixed base.

It, therefore, seems improbable that the renal ammonia mechanism has anything to do with the regulation of the acid-base balance of the body. The evidence at hand appears to be more consistent with the hypothesis that secretion of ammonia is stimulated by, and serves to neutralize, the acid residue left in the tubules by reabsorption of the alkaline threshold moiety.

REFERENCES

1. Nash, T. P., and Benedict, S. R.: The Ammonia Content of the Blood, and Its Bearing on the Mechanism of Acid Neutralization in the Animal Organism, J. Biol. Chem. **48**: 463, 1921.
2. Hendrix, B. M., and Sanders, J. P.: The Effect of Injections of Sodium Phosphates and Sodium Hippurate Upon the Excretion of Acid and Ammonia by the Kidney, J. Biol. Chem. **58**: 503, 1923.
3. Cushny, A. R.: The Secretion of the Urine, London, 1917, Longmans, Green & Co.
4. Briggs, A. P.: Excretion of Ammonia and Neutrality Regulation, J. Biol. Chem. **104**: 231, 1934.
5. Hendrix, B. M., and Calvin, D. B.: The Loss of Bases in Diuresis and Its Effect Upon the Alkali Reserve of the Blood, J. Biol. Chem. **65**: 197, 1925.
6. Carr, A. D.: The Effect of Water Diuresis on the Elimination of Certain Urinary Constituents, J. Pharmacol. & Exper. Therap. **18**: 221, 1921.
7. Barnett, G. D., and Blume, F. E.: Alkaline Tides, J. Clin. Investigation **17**: 152, 1938.
8. Briggs, A. P.: The Acidosis of Nephritis; Its Clinical Significance, Arch. Int. Med. **49**: 56, 1932.
9. Linder, G. C.: The Effect of Mineral Acid on Acid-Base Regulation in Health and in Nephritis, Quart. J. Med. **20**: 285, 1927.
10. Gamble, J. L., Blackfan, K. D., and Hamilton, B.: A Study of the Diuretic Action of Acid Producing Salts, J. Clin. Investigation **1**: 359, 1925.
11. Smith, Homer, W.: The Physiology of the Kidney, New York, 1937, Oxford Univ. Press.
12. Walker, A. M.: Ammonia Formation in the Amphibian Kidney, Am. J. Physiol. **131**: 187, 1940.
13. Walker, A. M., Hudson, C. L., Findley, I., Jr., and Richards, A. N.: The Total Molecular Concentration and the Chloride Concentration of Fluid From Different Segments of the Renal Tubule of Amphibia, Am. J. Physiol. **118**: 121, 1937.
14. Montgomery, H., and Pierce, J. A.: The Site of Acidification of the Urine Within the Renal Tubule in Amphibia, Am. J. Physiol. **118**: 144, 1936.
15. Walker, A. M., and Bott, Phyllis, A., Oliver, Jean, and MacDowell, Muriel C.: The Collection and Analysis of Fluid From Single Nephrons of the Mammalian Kidney, Am. J. Physiol. **134**: 580, 1941.
16. Ellinger, P.: Site of Acidification of Urine in Frog's and Rat's Kidney, Quart. J. Exper. Physiol. **30**: 205, 1940. (Brit. Chem. & Physiol. Ab. A, III.—16, January, 1941.)

BROMSULPHALEIN RETENTION IN LOW-GRADE CHRONIC ILLNESS

Major M. H. Stiles, M.C., U. S. Army, M. T. Stiles, and A. McM. Kolb
Philadelphia, Pa.

LIVER dysfunction has been reported associated with a variety of chronic or recurrent conditions; e.g., allergy,[1-4] "rheumatism" and arthritis,[5-11] peptic ulcer,[12] glaucoma,[13] Parkinsonism,[4] thyrotoxicosis,[14-17] rheumatic fever,[18] various infections and bacterial intoxication.[4, 12, 19-23] Because of these reports liver function studies were made on a group of patients with low-grade chronic illness probably secondary to chronic infection.

The clinical condition, sedimentation rate, and nonfilament-filament ratio,[24, 25] incidence of probable pathogenic streptococci and staphylococci[26] and distribution of metabolic rates[27] of this group have been reported. One hundred and twelve ambulant patients, varying in age from 15 to 82, were included. They were classified according to age, sex and the severity of objective symptoms.

In the choice of a method for determining liver function the bromsulphalein test was considered favorably, since it could be performed in an office on ambulant patients, it did not consume much of the patient's time, and it was considered by many to be the most generally satisfactory test in the absence of jaundice.[28-31] However, while some[32, 33] felt that the usual bromsulphalein test was very sensitive and would indicate early, and perhaps even temporary liver dysfunction, others[5, 34, 35] felt that it was necessary to devise an even more delicate test for use in mild cases. Macdonald,[36, 37] who studied this question intensively, pointed out that because of the great liver reserve a normal result with the usual bromsulphalein test did not indicate an absence of hepatic damage, although an abnormal retention almost definitely meant a diseased liver. Studying liver reserve in the search for a test which would detect minor grades of impairment, he developed a technique by means of which the blood could be examined at two-minute intervals following the injection of bromsulphalein. With this method he observed that the rate of bromsulphalein removal from the blood of normal persons followed a definite curve, and that disease of the liver produced not only a retention of bromsulphalein beyond the usual test period, but also a change in the type of curve. As a result of his observations Macdonald concluded that a quantity of bromsulphalein equivalent to 5 mg. per kilogram of body weight was necessary to test fully the liver reserve, that the normal undamaged liver would completely free the blood of this quantity of bromsulphalein in twenty-five minutes, and that the maximum information about liver function would only be obtained if the blood level of bromsulphalein were determined every five minutes. In a recent

Received for publication, July 11, 1942.

evaluation of liver function tests Mateer and his colleagues[38] found Macdonald's serial bromsulphalein test sensitive and reliable, even though the 2 mg. per kilogram dose was mainly used. Suggestions for fractional tests of liver function have also been made by White, Deutsch, and Maddock,[39] and by Deutsch.[40]

TABLE I

STATISTICAL ANALYSIS OF BROMSULPHALEIN RETENTION DISTRIBUTED ACCORDING TO SEX, AGE, SEVERITY OF SYMPTOMS AND SEDIMENTATION RATE

GROUP	NO.	RANGE	MEAN	±	S.E.	% 0	% 5-20	% 20+
All patients	112	0-90	18.93	±	1.63	14.3	56.3	29.3
Sex								
Male	45	0-70	19.33	±	2.45	15.6	51.1	33.3
Female	67	0-90	18.66	±	2.18	13.4	59.7	26.9
Age								
Up to 20	9	0-60	18.33	±	5.84	11.1	66.6	22.2
21-40	27	0-50	12.78	±	2.4	25.9	52.0	22.1
41-60	49	0-90	17.96	±	2.54	12.2	65.4	22.4
61+	27	0-70	27.04	±	3.4	7.4	40.8	51.8
Symptoms								
Mild	48	0-30	10.0	±	1.19	22.9	70.9	6.2
Moderate	47	0-50	18.94	±	1.83	10.6	53.2	36.2
Severe	17	5-90	44.71	±	5.25	0.0	23.5	76.5
Sedimentation rate in mm. (Westergren)								
1-10	59	0-50	14.75	±	1.71	20.4	57.5	22.1
11-20	28	0-90	20.71	±	3.89	10.7	57.1	32.2
21-30	13	0-60	22.31	±	4.68	7.7	53.9	38.4
31+	8	5-70	32.50	±	7.39	0.0	37.5	62.5

No. = Number of patients in group. Range = range of bromsulphalein retention in units. S.E. = Standard error. % 0 = per cent of patients in group with no bromsulphalein retention. % 5-20 = per cent with 5 to 20 units retention. % 20 + = per cent with more than 20 units retention.

In the present study Macdonald's normal criterion of complete elimination in twenty-five minutes of a dose of bromsulphalein equal to 5 mg. per kilogram body weight was adopted. The estimated quantity of bromsulphalein solution diluted with an equal quantity of physiologic salt solution was slowly injected with a No. 26 gauge needle. For simplicity usually only a single specimen of blood was withdrawn twenty-five minutes later. The use of larger needles and of full strength bromsulphalein solution in early tests was occasionally followed by thrombophlebitis, which in one instance was moderately severe and resulted in several days' incapacitation.

RESULTS

A statistical analysis of the results of the bromsulphalein tests is presented in Table I. Of the 112 patients only 16 showed no retention of bromsulphalein at the end of twenty-five minutes. If the blood had been examined at five-minute intervals throughout the test period, it is probable that even some of these would have shown abnormal curves.[41] Somewhat more than half of the total number showed bromsulphalein retention of from 5 to 20 units. The remaining patients showed higher degrees of retention, the highest being 90 units. The mean value for the entire group was 18.9. The frequency distribution of the tests is illustrated in Fig. 1.

When the observations were distributed according to sex, very little difference was found, the mean bromsulphalein retention being 19.3 units for males and 18.7 units for females (Fig. 2). When the observations were distributed according to age, greater differences were noted (Fig. 2). The mean bromsulphalein retention in units for patients up to 20 was 18.3; 21 to 40, 12.8; 41 to 60, 17.0; and 61 and over, 27.0. The mean rates for patients 21 to 40, and for those 61 and over, varied significantly from the mean of the entire group.

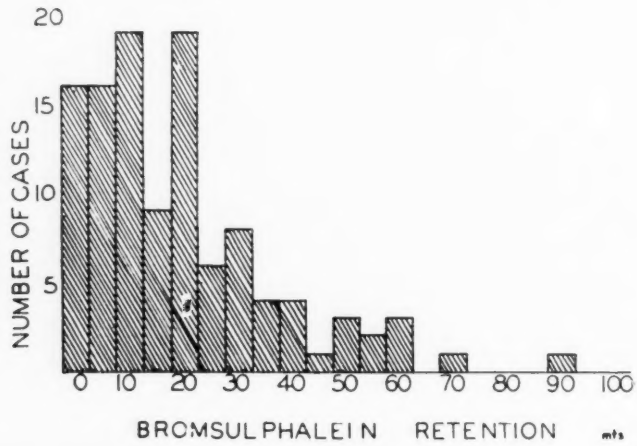


Fig. 1.—Frequency distribution of bromsulphalein retention in 112 patients with low-grade chronic illness.

When the observations were distributed according to the severity of symptoms (Fig. 3), greater differences appeared. The mean bromsulphalein retention in patients with mild symptoms was 10 units; in patients with moderately severe symptoms, 18.9 units; and in patients with severe symptoms, 44.7 units. Of the patients with mild symptoms 22.9 per cent showed no retention of bromsulphalein and only 6.2 per cent showed retention of more than 20 units. Of those with severe symptoms all showed some retention and 76.5 per cent showed retention of more than 20 units.

Since it has been suggested that there may be a relation between liver function and the sedimentation rate of erythrocytes,[42] the bromsulphalein retention and the sedimentation rate (Westergren) were compared (Fig. 4). The mean bromsulphalein retention for patients with sedimentation rates, ranging from 1 to 10 mm., was 14.75 units; 11 to 20 mm., 20.7 units; 21 to 30 mm., 22.3 units; and over 30 mm., 32.5 units. Yet, while in general higher sedimentation rates were associated with higher degrees of bromsulphalein retention, the association was far from complete. For example, in patients with sedimentation rates up to 10 mm. the degree of bromsulphalein retention varied from 0 to 50 units, and in those with sedimentation rates of 31 mm. or higher it varied from 5 to 70 units. The lack of a definite linear relation between sedimentation rate and bromsulphalein retention is illustrated by the scatter diagram in Fig. 5.

The differences between the mean bromsulphalein retention rates of patients with mild, moderately severe, and severe symptoms were all statistically significant, as were also the variations in relation to changes in sedimentation rate.

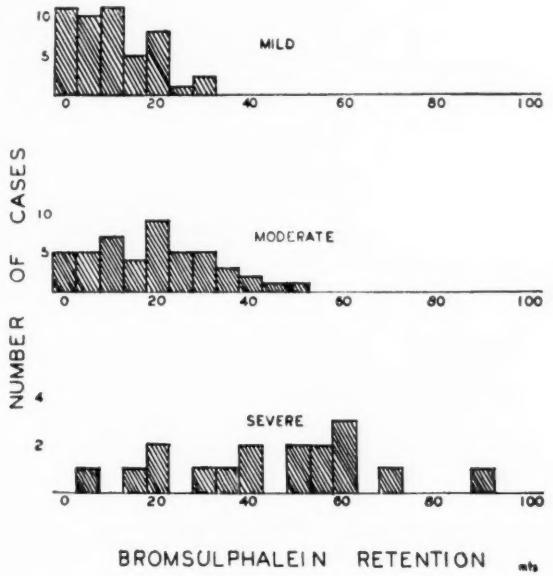


Fig. 2.—Bromsulphalein retention distributed according to sex and age.

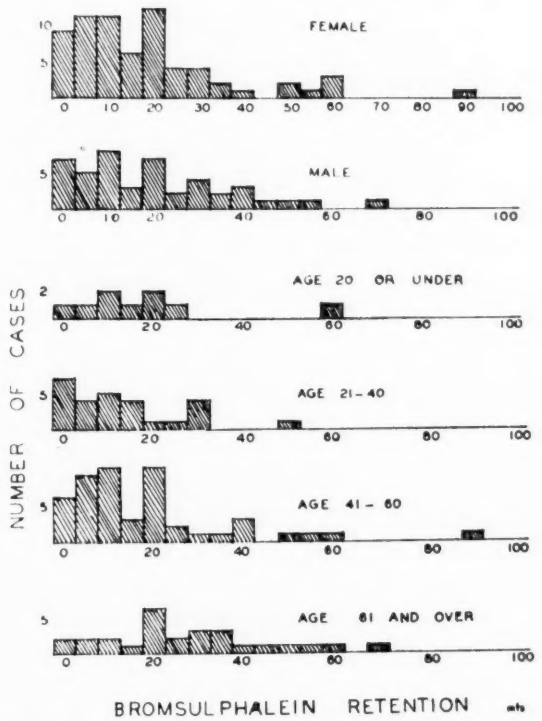


Fig. 3.—Bromsulphalein retention distributed according to severity of general symptoms.

COMMENT

The high incidence of impaired liver function in persons with chronic illness should not cause surprise if the reports of earlier writers are recalled. Rehfuss[43] has stated that biliary tract disturbance is probably present in one half of all persons past the age of 40. MacLagan and Rundle[17] stated that of 41 patients with hyperthyroidism, 30 showed definite impairment of hepatic function and the remaining 11 were border line. Shaffer,[16] in examining the livers of persons who died accidental deaths, found that 31 of 50 showed fatty infiltration, and 11 of 50 showed chronic localized interstitial hepatitis. Rosenberg[44] used the cephalin-cholesterol flocculation test in studying a group of 155 persons of whom 83 had clinical evidence of liver disease. He found liver impairment in practically all of those with clinical liver disease and in 43 of

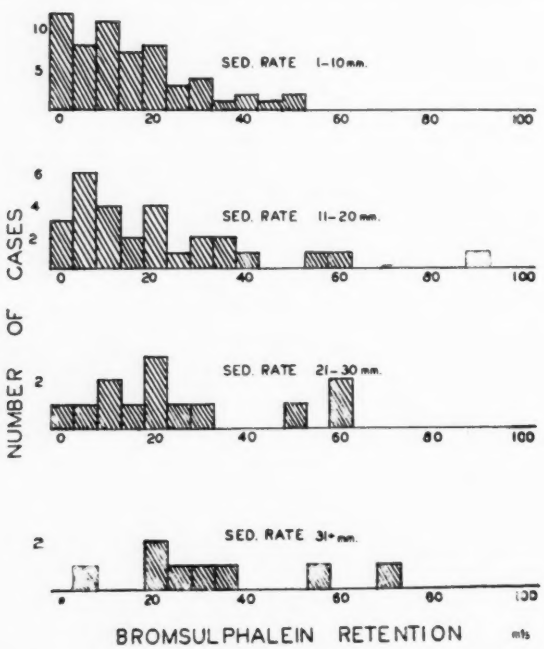


Fig. 4.—Frequency distribution of bromsulphalein retention in relation to sedimentation rate.

the 72 in whom hepatic disease was not suspected. He concluded that mild and subclinical disease of the liver occurs more frequently than is generally appreciated, and unless specifically sought may elude recognition. Apparently liver disturbance is frequent even in early life, since Londe and Probstein[45] found evidence of disturbed liver function in two children whose only departure from health had been recent upper respiratory infections, and in 9 of 13 children with histories of repeated colds. These findings are consistent with the observations made in the present study, where 8 of 9 children and adolescents showed some degree of bromsulphalein retention.

While the variations in bromsulphalein retention in relation to age are statistically significant, their actual significance is somewhat impaired by the high proportion of patients with mild symptoms in the age group 21 to 40, and

the high proportion with severe symptoms in the age group 61 and over. It may be significant that in the series of studies which have been made on patients with low-grade chronic illness,[24, 26, 27] the group from 21 to 30 years of age showed fewer deviations from the normal than did any other group.

The variations in bromsulphalein retention in relation to severity of symptoms also are consistent with the observations made in other studies of low-grade chronic illness. Patients with mild symptoms showed the least deviation from normal in the sedimentation rate,[24] the nonfilament-filament ratio,[24] the incidence of pathogenic streptococci,[26] and in the basal metabolic rate;[27] and persons with severe symptoms showed the greatest deviation from normal in all of these tests. Rawls, Weiss, and Collins[8] noted that liver dysfunction occurred with greater frequency in patients with severe than in those with mild rheumatoid arthritis.

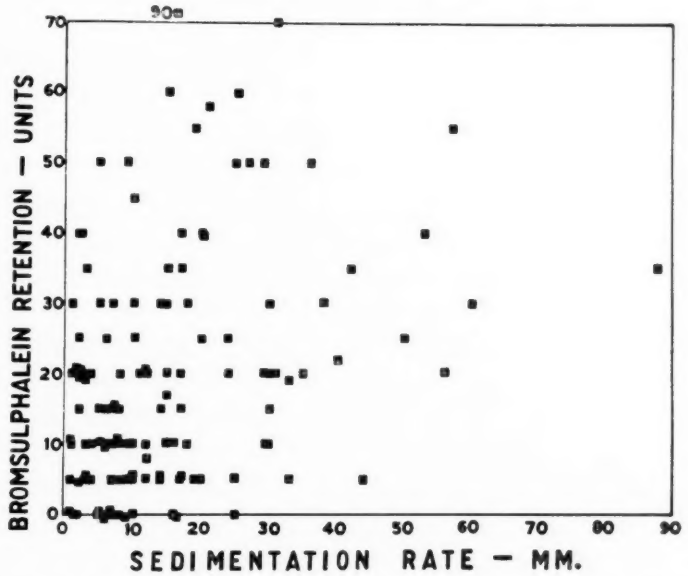


Fig. 5.—Relation of bromsulphalein retention (units) and sedimentation rate (mm. in one hour, Westergren) in individual patients.

While patients with high sedimentation rates had significantly greater bromsulphalein retention than did those with low sedimentation rates, it is possible that abnormal results with both tests are related more to the severity of illness than to each other. In view of the high degree of bromsulphalein retention occasionally associated with a practically normal sedimentation rate, and the high sedimentation rates associated with little bromsulphalein retention, there does not seem to be a direct relation between the two tests. It is possible, of course, that some other test of liver function might give results more nearly parallel to the sedimentation rate. It seems more probable, however, that, as Wintrobe[46] has said of the sedimentation rate, neither the sedimentation rate nor the bromsulphalein retention should be regarded as a precise, quantitative measurement, but should be accepted as a general indication of somatic or hepatic disturbance.

SUMMARY

Studies of bromsulphalein retention were made on 112 patients with low-grade chronic illness, using a dose of 5 mg. per kilogram of body weight.

On the basis of Macdonald's criterion of normal excretion all but 16 showed evidence of liver dysfunction. Of the 96 with dysfunction 33 showed more than 20 units retention of bromsulphalein.

The mean bromsulphalein retention of males differed little from that of females. The mean retention of patients aged 21 to 40 years was less than the general average and that of patients over 60 was greater, but the actual significance is uncertain.

When the mean bromsulphalein retention of patients with mild general symptoms was compared with the mean retention of patients with moderately severe and severe symptoms, statistically significant differences were found, the respective values being 10.0, 18.9 and 44.7 units.

While patients with high sedimentation rates tended to have a high degree of bromsulphalein retention, there were a number with high rates who had little retention, and a number with low rates who had considerable retention.

CONCLUSIONS

Impairment of liver function is found in a considerable proportion of patients with low-grade chronic illness.

The degree of impairment varies directly with the severity of symptoms.

REFERENCES

1. Shay, H., Gersohn-Cohen, J., and Fels, S. S.: The Factor of Occult Hepatic and Biliary Tract Disease in Some Cases of Allergy, Am. J. Digest. Dis. **6**: 335, 1939.
2. Schmengler, F. E.: Hepatic Cirrhosis and Hepatargy on Allergic Basis, Klin. Wchnschr. **19**: 1155, 1940.
3. Coke, F., and Coke, H.: Asthma, Baltimore, 1939, Williams & Wilkins Co.
4. Todd, A. T.: Treatment of Some Chronic and Incurable Diseases, Bristol, 1937, John Wright & Sons Ltd.
5. Hench, P. S., Bauer, W., Dawson, M. H., Hall, F., Holbrook, W. P., Key, J. A., and McEwen, C.: The Problem of Rheumatism and Arthritis, Ann. Int. Med. **14**: 1383, 1941.
6. Burbank, R.: An Outline of the Aetiology, Pathology and Treatment of Arthritis, Tri-State M. J. **12**: 2355, 1939.
7. Collins, V. L.: The Relation of Liver and Gallbladder Disease to Arthritis, Rev. Gastroenterol. **6**: 344, 1939.
8. Rawls, W. B., Weiss, S., and Collins, V. L.: Liver Function in Rheumatoid Arthritis, Ann. Int. Med. **10**: 1021, 1937.
9. Rawls, W. B., Weiss, S., and Collins, V. L.: Liver Function in Rheumatoid Arthritis, Ann. Int. Med. **12**: 1455, 1939.
10. Peers, R. S.: Gallbladder: Its Relation to Chronic Arthritis of Uncertain Etiology, California & West. Med. **51**: 374, 1939.
11. Andrews, K. R., and Muether, R. O.: Effect of Diet on Arterial and Venous Glucose Tolerance Curve in Rheumatoid Arthritis, J. Lab. & Clin. Med. **26**: 675, 1941.
12. Weir, J. F.: Hepatitis: Some Forms Not Commonly Recognized, J. A. M. A. **111**: 1356, 1938.
13. Schmelzer, H.: Examination of Hepatic Function in Patients With Glaucoma, Arch. f. Ophth. **141**: 266, 1939.
14. Haines, S. F., Magath, T. B., and Power, M. H.: The Hippuric Acid Test in Hyperthyroidism, Ann. Int. Med. **14**: 1225, 1941.
15. Lichtmann, S. S.: Liver Function in Hyperthyroidism, With Special Reference to the Galactose Tolerance Test, Ann. Int. Med. **14**: 1199, 1941.
16. Shaffer, J. M.: Disease of Liver in Hyperthyroidism, Arch. Path. **29**: 20, 1940.
17. MacLagan, N. F., and Rundle, F. F.: Liver Function in Thyrotoxicosis, Quart. J. Med. **9**: 215, 1940.

18. Lichtmann, S. S.: Cinchophen Oxidization Test of the Function of the Hepatic Cells, Arch. Int. Med. **48**: 98, 1931.
19. Schottmuller, H.: Hepatitis and Cholecystitis, Specific Toxic Complication of Scarlet Fever, Klin. Wchnschr. **10**: 17, 1931.
20. Steidl, J., and Heise, F. H.: Studies of Liver Function in Advanced Pulmonary Tuberculosis, Am. J. M. Sc. **186**: 631, 1933.
21. Cates, H. B.: Relation of Liver Function to Cirrhosis of Liver and to Alcoholism, Arch. Int. Med. **67**: 383, 1941.
22. Portis, S. A.: Hepatitis: Etiology, Diagnosis and Treatment, New Orleans M. & S. J. **92**: 415, 1940.
23. McNee, J. W.: Hepatitis, Edinburgh M. J. **46**: 305, 1939.
24. Stiles, M. H.: Sedimentation Rate and Nonfilament-Filament Ratio in Low Grade Chronic Illness, Arch. Int. Med. **63**: 664, 1939.
25. Stiles, M. H.: Changes in Sedimentation Rate and Nonfilament-Filament Ratio Following Clinical Improvement in Patients With Low Grade Chronic Illness, J. Lab. & Clin. Med. **26**: 621, 1941.
26. Stiles, M. H., and Chapman, G. H.: Probable Pathogenic Streptococci and Staphylococci in Chronic Low Grade Illness, Arch. Otolaryng. **31**: 458, 1940.
27. Stiles, M. H.: Basal Metabolic Rate in Low Grade Chronic Illness. A Statistical Analysis of 166 Cases, Am. J. Clin. Path. **11**: 871, 1941.
28. Gerendasy, J.: An Evaluation of Liver Function Tests, Rev. Gastroenterol. **7**: 186, 1940.
29. Newell, T. E., and Hoerner, M. T.: The Diagnosis and Treatment of Jaundice, Ohio State M. J. **36**: 365, 1940.
30. Snell, A. M., and Magath, T. B.: The Use and Interpretation of Tests for Liver Function, J. A. M. A. **110**: 167, 1938.
31. Editorial: The Effect of Anesthetics on Hepatic Function, J. A. M. A. **110**: 1755, 1938.
32. Israel, N. L., and Reinhold, J. G.: Detection of Cirrhosis and Other Diseases of the Liver by Laboratory Tests, J. Lab. & Clin. Med. **23**: 588, 1938.
33. DeLor, C. J., and Reinhart, H. L.: An Analysis of the Hippuric Acid, Galactose Tolerance, Bromsulphalein and Prothrombin Tests in 381 Cases, Am. J. Clin. Path. **10**: 617, 1940.
34. Morrison, L. M., and Swalm, W. A.: New Physiologic Test for Liver Function: Preliminary Report, Rev. Gastroenterol **7**: 269, 1940.
35. Rosenberg, D. H., and Soskin S.: The Azorubin S Test for Liver Function: An Evaluation, With a Comparative Study of the Bromsulphalein and Hippuric Acid Tests, Ann. Int. Med. **13**: 1645, 1940.
36. Macdonald, D.: Some Observations on Disappearance of Bromsulphalein Dye From the Blood: Its Relation to Liver Function (Preliminary Report), Canad. M. A. J. **39**: 556, 1938.
37. Macdonald, D.: A Practical and Clinical Test for Liver Reserve, Surg., Gynec. & Obst. **69**: 70, 1939.
38. Mateer, J. G., Baltz, J. L., Marion, D. F., Hollands, R. A., and Yagle, E. M.: A Comparative Evaluation of the Newer Liver Function Tests, Am. J. Digest. Dis. **9**: 13, 1942.
39. White, F. W., Deutsch, E., and Maddock, S.: The Comparative Value of Serial Hippuric Acid Excretion, Am. J. Digest. Dis. **6**: 603, 1939.
40. Deutsch, E.: A Fractional Bromsulphalein Test to Determine Liver Damage in the Nonjaundiced Patient, New England J. Med. **225**: 171, 1941.
41. Macdonald, Dean: Personal communication.
42. Lichtmann, S. S.: The Influence of Intravenous Glucose Injections on Abnormal Erythrocyte Sedimentation Speed in Relation to Activity of Infection, Ann. Int. Med. **13**: 1297, 1940.
43. Rehfuss, M. E.: The Management of the Gallbladder Patient, Pennsylvania M. J. **42**: 1335, 1939.
44. Rosenberg, D. H.: Cephalin-Cholesterol Flocculation Test in Cases of Disease of Liver, With Special Reference to Diagnosis of Mild and Unsuspected Forms, Arch. Surg. **43**: 231, 1941.
45. Londe, S., and Probstein, J. G.: Hippuric Acid Liver Function Test in Children, J. Pediat. **18**: 371, 1941.
46. Wintrobe, M. M.: The Erythrocyte Sedimentation Test, Am. J. Clin. Path. **11**: 562, 1941.

113 W. Chestnut Hill Avenue

SOLUBILITY AND pH DATA OF SOME OF THE COMMONLY USED SULFONAMIDES*

WILLIAM G. CLARK, PH.D., ERNEST A. STRAKOSCH, M.D., M.S., AND NATHAN I. LEVITAN, M.S., MINNEAPOLIS, MINN.

WHILE working in the field of the local application of sulfonamides to infections, we frequently needed solubility and pH data of the sulfonamides. A search of the literature has failed to reveal a source of collected data on these physical properties of the more commonly used sulfonamides. The data which exist are very scattered and frequently appear in papers which are abstracted in such a way as to disguise the fact that such data do appear in the papers. Moreover, some data are lacking in the literature we have been able to examine, including brochures and pamphlets of various commercial firms.

Hence it was thought that if these data were made available in collected form in a single table, it would prove extremely useful to workers in the fields of chemotherapy, particularly at the present time. For this reason, we have determined the solubilities of the commonly used sulfonamides and their sodium salts at room temperature and at body temperature in water and in human serum. In addition, we have determined the pH values of the saturated aqueous solutions, since this information is also lacking in many instances.

EXPERIMENTAL

A small tinted glass container containing excess sulfonamide in carbon dioxide-free distilled water or in serum, was shaken in a water bath thermostatically controlled to within 0.1° C. for twenty-four hours. The saturated solution was then filtered by aspiration through a washed and dried asbestos filter stick into a weighed weighing bottle. The entire apparatus was kept at the temperature at which the compound was dissolved (25° C. or 37° C.). The amount dissolved was then determined by the method of Bratton and associates,[1] using a photoelectric colorimeter. The pH values in carbon dioxide-free distilled water were determined at 25° C. with a Coleman pH meter (glass electrode/calomel electrode). The results are tabulated in Table I, along with values collected from the literature by other workers.

The large difference in solubility of sulfathiazole and sulfadiazine in water versus serum are striking, especially the latter. It has been pointed out by Roblin and others,[8] that blood levels exceed the solubility of sulfadiazine in water, while the blood levels obtained with the other sulfonamides are below the water solubility.

*From the Division of Physiology, Department of Zoology, and the Division of Dermatology and Syphilology, University of Minnesota, Minneapolis.

Supported by grants from the General Research Fund of the University of Minnesota, and the Abbott Laboratories, North Chicago, Ill. Assistance was furnished by the personnel of Work Projects Administration, O.P. 165-1-71-124, Subproject 383.

Received for publication, July 15, 1942.

TABLE I

SOLUBILITY AND pH DATA OF SOME OF THE COMMONLY USED SULFONAMIDES
(% = grams per 100 Gm. of the solvent)

COMPOUND	25° C. TEMPERATURE					37° C. TEMPERATURE			
	WATER (%)	HUMAN SERUM (%)	pH*	REFS.	REMARKS	WATER (%)	HUMAN SERUM (%)	REFS.	REMARKS
Sulfanilamide	0.836	0.981	7.15	L†		1.460	1.970	L	
	0.750	-----	-----	7		1.500	-----	4	
Sulfathiazole	0.0502	0.234	5.90	L	At 26° C.	0.0960	0.330	L	
	0.0600	-----	-----	2		0.0942	-----	10	
						0.0940	-----	8	
						-----	0.184	4	At 36° C.
Sulfapyridine	0.0268	0.053	6.65	L		0.0486	0.0756	L	
	0.0280	-----	-----	7		0.0495	-----	5	
						0.0490	-----	7	
						0.0530	-----	9	
						-----	0.0610	4	At 36° C.
Sulfadiazine	0.0077	0.115	5.75	L		0.0127	0.161	L	
						-----	0.124	4	At 36° C.
						0.0123	-----	8	
						0.0120	0.107**	10	
						-----	0.247***	10	
Sodium sulfathiazole	45.0		9.65****	L	Coagulates serum	60.0		L	Coagulates serum
	45.0			3					
Sodium sulfapyridine	52.0		10.85	L	Coagulates serum	80.0		L	Coagulates serum
	54.0			6					
Sodium sulfadiazine	50.0		9.90	L	Coagulates serum	65.0		L	Coagulates serum

*All pH determinations were made in carbon dioxide-free water solutions.
†L refers to our determinations. The numbers refer to those of others.
**Horse serum.
***Defibrinated rabbit blood.
****The pH values for 0.01 molar aqueous solutions were found to be:

Sodium sulfathiazole	pH 9.20
Sodium sulfapyridine	pH 10.10
Sodium sulfadiazine	pH 8.75

We are at present determining similar data for the sulfonamides in other solvents which may be of more practical use in the topical treatment of infections.

REFERENCES

1. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.
2. Lott, W. A., and Bergeim, F. H.: (p-Aminobenzenesulfonamido)-Thiazole: A New Chemotherapeutic Agent, J. Am. Chem. Soc. **61**: 3593, 1939.
3. Pharmacological Activity and Clinical Use of Sulfathiazole, Merck & Co., Inc., Rahway, N. J., April, 1942, page 1.
4. Hawking, F.: Local Concentration of Sulphonamide Compounds Inserted Into Wounds, Lancet **240**: 786, 1941.
5. Hug, E.: Solubility of Sulfapyridine and Acetylsulfapyridine Under Various Conditions, Rev. Soc. argent. de biol. **16**: 662, 1940.
6. Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., Jr.: The Toxicity and Absorption of 2-Sulfanilamidopyridine and Its Soluble Sodium Salt, Science **88**: 597, 1938.
7. Clinical Use of Sulfanilamide Compounds in the Treatment of Gonococcal Infections, Merck & Co., Inc., Rahway, N. J., Nov., 1941, pp. 1-3.
8. Roblin, R. O., Jr., Williams, J. H., Winnek, P. S., and English, J. P.: Chemotherapy. II. Some Sulfanilamido Heterocycles, J. Am. Chem. Soc. **62**: 2002, 1940.
9. Roblin, R. O., Jr., and Winnek, P. S.: Chemotherapy. I. Substituted Sulfanilamidopyridines, J. Am. Chem. Soc. **62**: 1999, 1940.
10. Feinstone, W. H., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L.: The Toxicity, Absorption and Chemotherapeutic Activity of 2-Sulfanilamidopyrimidine (Sulfadiazine), Bull. Johns Hopkins Hosp. **67**: 427, 1940.

FAT METABOLISM IN ACNE VULGARIS*

EDWARD B. LEWINN, M.D., AND I. ZUGERMAN, M.D.
PHILADELPHIA, PA.

WHILE the relationship between fat metabolism and acne vulgaris has been suggested previously,[1] no studies of this problem have been made by methods which reflect the status of the fat metabolism in this skin disease. It has also been proposed that acne vulgaris is of endocrine origin, but up to the time of the present report the investigations have been disappointing, the results permitting no certain conclusions. Cholesterol is chemically related to the steroidal hormones, such as estrogens and androgens, the latter having been found to cause acne when given to women in very large doses.[2] On the other hand, cholesterol is intimately associated with the metabolism of fats. This dual relationship was utilized in a study of fat tolerance in acne vulgaris, herein reported, employing the blood cholesterol as an indicator of the blood lipid content.[3]

MATERIAL

In this investigation 20 patients, 12 females and 8 males, with acne vulgaris were studied. Their ages ranged from 11 to 29 years, the average being 19 years. The duration of their acne varied from one month to 11 years. The severity of the lesions varied from a few well-marked comedones and pustules on the forehead to extensive and active involvement of the forehead, face, shoulders, chest, and back. Most of the 12 female patients exhibited some menstrual disturbances, mainly irregularity or amenorrhea.

Thirteen patients without evidence of past or present acne were studied for comparison with the acne patients. These patients also served as controls in a study of fat metabolism in psoriasis.[3] There were 8 women and 5 men in the group, ranging in age from 20 to 65 years, the average being 41 years. The average age of the control group is considerably higher than that of the acne patients. However, advancing age causes only very slight changes in blood cholesterol levels.[4]

PROCEDURE

After a twelve-hour fast a blood specimen was taken. The patient then drank 500 c.c. of 20 per cent cream, representing 100 Gm. of fat. Blood specimens were then obtained at intervals of one and one-half, three, four and one-half, six, and seven and one-half hours, and the whole blood was examined for total cholesterol by the method of Bloor.[5] During the time of the test each patient was allowed to take an occasional sip of water. Most patients were ambulant but activity was closely restricted.

*From the Medical Service of Dr. Mitchell Bernstein and the Dermatological Service of Dr. A. Strauss, Jewish Hospital, Philadelphia.
Received for publication, August 7, 1942.

RESULTS

The fasting total cholesterol levels in 20 cases of acne vulgaris were between 125 mg. and 275 mg. per 100 c.c. of whole blood, the mean being 173 mg. In the 13 control cases the fasting cholesterol ranged from 123 to 250 mg. per 100 c.c.,

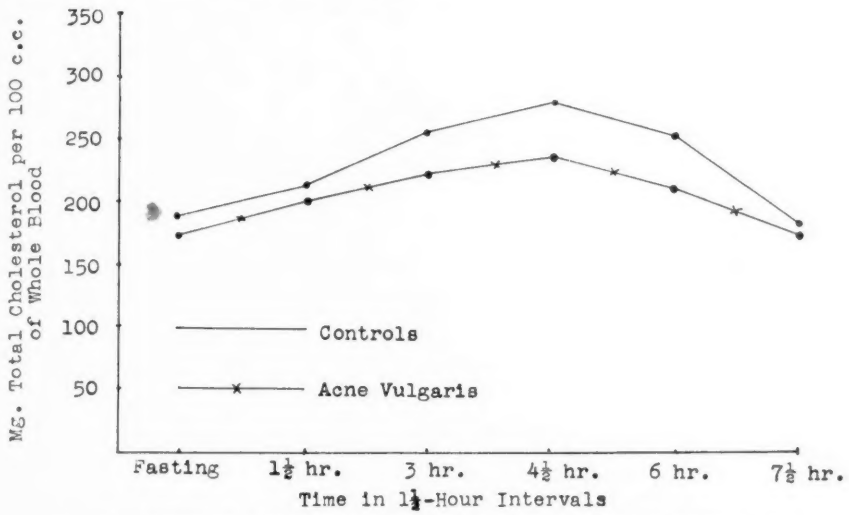


Fig. 1.—Composite blood cholesterol curves following the ingestion of fat.

the mean being 188 mg. In the acne patients the range of increases from fasting to the highest level of blood cholesterol was from 25 mg. to 208 mg. per 100 c.c. of whole blood, the mean being 83 mg. On the other hand, the control group showed increases above the fasting level of from 42 mg. to 198 mg., with a mean of 119 mg. (Table I).

TABLE I

PATIENTS WITH ACNE VULGARIS

CASE	FASTING	1½ HR.	3 HR.	4½ HR.	6 HR.	7½ HR.	MG. RISE
1	125	132	150	192	227	150	102
2	125	135	151	156	147	131	31
3	132	150	172	185	178	150	53
4	139	156	166	166	178	208	69
5	147	166	172	192	217	178	70
6	150	178	208	238	192	166	88
7	150	172	192	200	217	172	67
8	151	172	178	185	208	160	57
9	160	208	250	227	175	160	90
10	160	192	222	227	208	178	67
11	166	189	227	189	172	151	61
12	166	156	171	191	166	138	25
13	166	191	250	263	227	171	97
14	166	178	208	238	192	166	72
15	192	208	217	227	250	208	58
16	208	227	250	263	238	192	55
17	208	250	312	416	208	178	208
18	227	263	295	333	250	192	106
19	250	333	257	455	357	312	205
20	275	257	312	227	184	166	82

Data for control patients have been presented in an earlier publication.[3]

Examination of the curves of the means of the two groups does not reveal a significant difference between them in fat tolerance, all points of the composite curves of both groups being within accepted normal limits. The marked similarity between the acne and the nonacne patients is further emphasized by statistical examination of the figures. The application of the t test to the fasting levels of blood cholesterol gives a value of 0.3. The value of t for the peak levels of the two groups is 0.6. For groups of the size studied in this report t should have a value of 2.04 or greater to be considered significant. It is worthy of note that Strickler and Adams[6] were also unable to demonstrate any departure from normal in the fasting blood cholesterol levels of acne patients.

CONCLUSIONS

A study of the fat tolerance, as indicated by blood cholesterol changes following the ingestion of fat, of a group of 20 patients with acne vulgaris fails to reveal any difference from a group of patients without acne vulgaris.

Dr. E. D. Burdick, of the University of Pennsylvania, assisted in the statistical review of our results.

Dr. O. E. Heim, Jr., permitted us to study several of his patients in the Dermatological Service at Frankford Hospital.

REFERENCES

1. Sutton, R. L., and Sutton, R. L., Jr.: Diseases of the Skin, ed. 10, St. Louis, 1939, The C. V. Mosby Co.
2. Geist, S. H., Salmon, U. J., Gaines, J. H., and Walter, R. T.: The Biologic Effects of Androgen (Testosterone Propionate) in Women, J. A. M. A. **114**: 1539, 1940.
3. LeWinn, E. B., and Zugerman, L.: Fat Tolerance Tests in Psoriasis, Am. J. M. Sc. **201**: 703, 1941.
4. Parhon, C. J., and Parhon, M.: L'hypercholesterinemie de la vieillesse, Compt. rend. Soc. de biol. **88**: 231, 1923.
5. Bloor, W. R.: The Determination of Small Amounts of Lipid in Blood Plasma, J. Biol. Chem. **77**: 53, 1928.
6. Strickler, A., and Adams, P. D.: Cholesterol Metabolism in Certain Dermatoses, With Special Reference to Acne Vulgaris, Arch Dermat. & Syph. **26**: 11, 1932.

LABORATORY METHODS

GENERAL

A MODIFICATION OF THE KAHN TEST FOR USE WITH HEMOLYZED BLOOD*

H. J. Lawler, Sc.D., St. Louis, Mo.

IN LABORATORIES which perform diagnostic tests for syphilis, one difficulty is that presented by blood specimen that has undergone so much hemolysis that precipitation tests cannot be read. The physician is usually notified that the specimen is not suitable for diagnostic test, and he is asked to send another sample of blood. There is inconvenience to both physician and patient.

Some idea as to the frequency of such hemolyzed specimens may be gained from the following tabulation of the last two years' experience of the St. Louis City Bacteriological Laboratory, where Kahn tests are performed on blood specimens collected by messenger from various points throughout the city each evening and tested the day after the blood is drawn.

	1940	1941
Total number of Kahn tests run	55,518	74,918
Number hemolyzed	343	689
Percentage hemolyzed†	59	91

The sharp increase in the number of hemolyzed specimens in the latter year is probably related to the fact that many of the specimens submitted in connection with the selective service physical examinations were drawn by physicians who had previously had relatively little experience with the submission of specimens to the municipal laboratory. Those physicians who send them in frequently are apparently more aware of the precautions which aid in the prevention of hemolysis.

When the percentages of hemolyzed specimens received by the laboratory are graphically represented in relation to the months of the year, as in Fig. 1, it is apparent that the problem is accentuated during the summer months. This was particularly true during 1941, when approximately four such specimens were received by the laboratory each working day from May through September.

*From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis.

†Included as hemolyzed are those specimens containing enough hemoglobin to obscure completely the reading of the Kahn test.

Received for publication, April 24, 1942.

The best solution of the problem would be the prevention of hemolysis, and much of it can be prevented by reminding the physician of the simple precautionary measures:

a. Use of a dry syringe and a fairly large needle.
b. Permit the blood to flow into the syringe, with a minimum of negative pressure.
c. Remove the needle from the syringe before *gently* emptying the syringe.
d. If feasible, place the specimen in a refrigerator, especially if there may be delay in getting it to the laboratory.

If, however, hemolyzed specimens are sent in, then the laboratory might well make use of them if adequate modification of existing methods is made available to assure the reliability of such tests. Any satisfactory method should remove the hemoglobin, should give the same results that would have been obtained with clear serum from the same source, and should be adapted to the equipment and skills of the usual public health laboratory.

The particular method described herein is essentially a modification of the usual Kahn test for spinal fluid. The serum is diluted with a relatively large volume of ammonium sulfate solution to "salt" out the globulin; this is centrifuged to a minimum volume. The supernatant solution, containing most of the hemoglobin of the original specimen, is poured off, the globulin is redissolved, and a slightly modified Kahn test is performed on the globulin solution.

MATERIALS AND METHODS

Syphilitic sera for testing the efficiency of the proposed method were obtained from the St. Louis City Bacteriological Laboratory. Only weakly positive and negative sera were used, for it was found that strongly positive sera gave uniformly positive tests even with less adequate modifications of the method and, therefore, could not be used in comparative evaluations. "Hemolyzed specimens" were prepared from these sera by the addition of "hemoglobin solution" prepared in the following manner: Packed red blood cells from nonsyphilitic blood were alternately frozen and thawed until hemolyzed. After removal of stroma by centrifugation, the resulting serum was adjusted to contain 20 Gm. of hemoglobin per 100 ml., as determined by the cyanmethemoglobin method in the Evelyn colorimeter (Evelyn and Malloy, 1938), and 0.2 ml. of this "hemoglobulin solution" was added to 0.8 ml. of serum of known syphilitic titer to produce 1 ml. of a hemolyzed specimen. These proportions were determined by estimation that hemolysis sufficient to obscure completely the Kahn precipitate might add 20 per cent to the volume of the serum.

The Kahn tests were performed in the manner prescribed by Kahn (1928). They were read immediately after the addition of saline, and again after fifteen minutes; the amounts of precipitate in the three tubes are expressed in the tables in this paper in terms of plus signs. The results of the second reading are recorded above the results of the first, if there was any change.

PREPARATION OF GLOBULIN SOLUTION

1. The specimen is subjected to centrifugation to remove cells, clot fragments, etc., as in the usual preparation of serum (fifteen minutes at 2,000 r.p.m.).

2. One milliliter of the supernatant fluid is pipetted off into a small round-bottomed test tube (the 100 by 12 mm. size is convenient), and after addition of 5 ml. of ammonium sulfate solution* the mixture is incubated at 56° C. for fifteen minutes.
3. The precipitate which is formed is separated by centrifuging at 2,000 r.p.m. for twenty minutes. Supernatant fluid is poured off, and the tube containing the precipitate is inverted over absorbent paper to remove as much of the salt solution as is convenient.
4. The sediment in the tube is dissolved by the addition of 1.3 ml. of distilled water, while stirring with a wooden applicator stick, and after centrifuging for five minutes at 1,500 r.p.m., the supernatant is transferred into another tube by means of a capillary pipette.
5. Three tube Kahn test is now set up, using 0.15 ml. of this globulin solution (instead of serum) and the usual amounts of antigen (Kahn, 1928) in each tube. The mixture is shaken at the rate of 275 times per minute for *four* minutes instead of three.

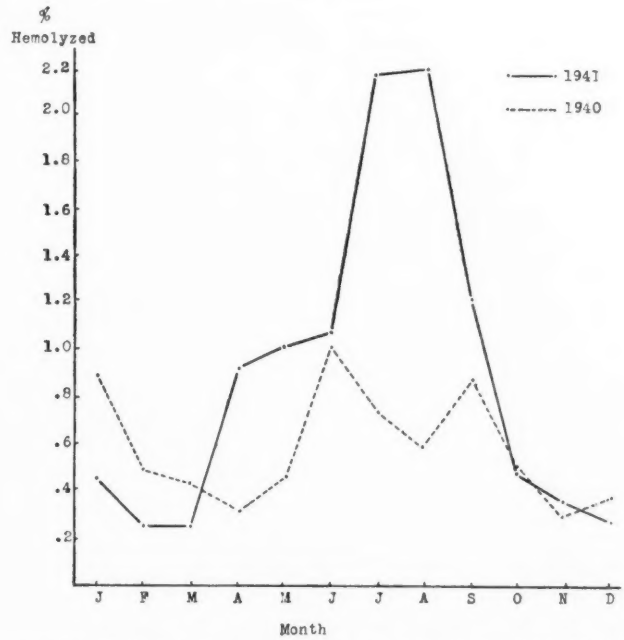


Fig. 1.—The occurrence of hemolyzed specimens among those received during 1940 and 1941 by the St. Louis Municipal Bacteriological Laboratory.

The reliability of this procedure was tested by using it with (a) "hemolyzed specimens" prepared as outlined previously, and (b) similar specimens were prepared by the addition of negative serum to the syphilitic sera in place of "hemoglobin solution." In Table I are listed the results of such tests, along with the results of standard Kahn tests run simultaneously on twenty-four different sera.

In general, these comparative tests showed good agreement between the standard Kahn and the modified test with globulin solution; the greatest varia-

*The solution is prepared by adding 48 Gm. of "analytical reagent" grade ammonium sulfate to 100 ml. of distilled water. In adding this to the serum, blowing it in is an easy method of mixing.

tion between the two in the case of any one serum was ++. The reagin-antigen precipitates were slightly smaller when formed from the globulin solution than when formed from whole serum, although the recommended extra minute's shaking given the antigen-globulin mixture tends to make this difference less pronounced.

An additional observation, possibly bearing on the evaluation of the modified test, was made in the case of globulin prepared from known negative sera: doubtful reactions (±) were frequent, and one-plus precipitates were formed in the middle tube in several cases. The particles of these precipitates were smaller and more evenly distributed than the usual Kahn precipitates.

TABLE I

RESULTS OF A MODIFIED KAHN TEST ON SOLUTIONS OF GLOBULIN COMPARED WITH THE RESULTS OF THE STANDARD KAHN TEST ON THE UNALTERED SERA

SERUM NO.	GLOBULIN PRECIPITATED FROM						CONTROL KAHN TEST ON ORIGINAL SERA		
	(A) 0.8 ML. POSITIVE SERUM + 0.2 ML. "HEMOGLOBIN SOLUTION"			(B) 0.8 ML. POSITIVE SERUM + 0.2 ML. NEGATIVE SERUM					
	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3
1	+++ +	++++	++++	++	++++	+++ +	++++	++++	++++
2	++ +	++++	++++	−	+++ +	+++ +	±	++++ +	++++
3	++ +	++++	++++	±	++++	+++ +	−	++++	+++ +
4	±	+++ +	+++ +	±	+++ +	+++ +	−	++	+++ +
5	−	±	±	−	+	−	−	−	−
6	−	+	−	−	±	±	−	−	−
7	−	+++	+++ +	−	+++	+++ +	−	++++	++++
8	−	+++	++++	−	+++	+++ +	−	+++	+++ +
9	−	++++	+++ +	−	+++	+++ +	−	++++	++++
10	+++	++++	+++ +	+++	+++ +	+++ +	++++	++++	++++
11	++++	++++	+++ +	++++	++++	+++ +	+++ +	++++	++++
12	++++	++++	+++ +	++++	+++ +	+++ +	+++ +	++++	++++
13	−	−	±	−	+	−	−	−	−
14	−	+	−	−	−	−	−	−	−
15	−	−	±	−	±	±	−	−	−
16	++	++++	+++ +				+++	++++	++++
17	++++	++++	++++				++++	++++	++++
18	++	+++ +	++++				−	++++	++++
19	+	++	++				−	±	+++
20	±	+	−				−	−	−
21	+	++++	+++ +				+	++	+++ +
22	++	++++	+++ +				+	++++	+++ +
23	−	±	−				−	−	−
24	−	−	−				−	−	−

FACTORS AFFECTING THE MODIFIED TEST

1. The amount of fluid used in dissolving the globulin.

Using four "hemolyzed specimens," prepared as outlined previously, parallel modified Kahn tests were run on solutions of globulin made by the addition of 0.8, 1.0, 1.2, and 1.4 ml. of 0.9 per cent saline solution to the precipitates. The results of these tests and of the simultaneously run standard Kahn tests on the original sera (Table II) showed the closest correspondence in the case

TABLE II

RESULTS OF THE MODIFIED KAHN TEST ON GLOBULIN SOLUTIONS MADE UP WITH VARIOUS AMOUNTS OF SALINE

SERUM NO.	AMOUNT OF SALINE ADDED TO GLOBULIN												CONTROL KAHN TEST ON ORIGINAL SERA		
	0.8 ML.			1.0 ML.			1.2 ML.			1.4 ML.					
	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3
1	++	++++	−	+++	++++	++++	+	+++					+		
2	++++	+++	−	++++	++++	+++	++	++++	++++	+	++++	++++	++	++++	++++
3	+++	++++	−	+ ++	+++ +++	++++	++++	++++ +	++++	+++	++++	++++	++++	++++ ++	++++
4	−	++++	++	∓	+++	+++	−	+++ ++	++++	−	+++	++++	+ ∓	++++ +	++++
5	+	++	++	−	+	++	−	±	±	−	±	−	−	−	−

TABLE III

RESULTS OF THE MODIFIED KAHN TEST ON SOLUTIONS OF GLOBULIN PRECIPITATED FROM SERA CONTAINING VARIOUS AMOUNTS OF HEMOGLOBIN

SERUM NO.	CONTENT OF SPECIMEN												CONTROL KAHN TEST ON ORIGINAL SERA		
	0.9 ML. SERUM + 0.1 ML. HB. SOLUTION			0.9 ML. SERUM + 0.2 ML. HB. SOLUTION			0.8 ML. SERUM + 0.3 ML. HB. SOLUTION			0.7 ML. SERUM + 0.4 ML. HB. SOLUTION					
	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3	TUBE 1	TUBE 2	TUBE 3
1	−	++ ++++	++++ 2	−	++ +++ ±	++++ 2	−	++	++++ 2	−	+++ ++	++++ 2	−	++ +++	++++ 2
2	−	++	++++ 2	−	++	++++ 2	−	+++	++++ 2	−	+++ ++	+++ ++ 2	−	± ++ ∓	++++ 2
3	−	++++	++++ 3	−	++++	++++ 3	−	+++ ±	++++ 2 ±	−	+++	+++ ++ 2	−	++++	++++ 3
4	−	−	− −	−	−	− −	−	±	± −	−	−	+ −	−		− −

of the solution made with 1.2 ml. of saline. More than that amount (1.4 ml.) seemed to make the test less sensitive, while less (1.0 ml.) made it more sensitive, negative serum number 5 showing a doubtful positive. Still less produced bizarre reactions (see Table II).

2. The effect of increased amounts of hemoglobin.

Four sets of "hemolyzed specimens" were prepared, containing different amounts of "hemoglobin solution," as outlined in Table III. Modified Kahn tests on globulin precipitated from these gave results very similar to those obtained with standard Kahn tests on the sera; the added hemoglobin apparently interfered little, except in the case of the specimens prepared with 0.4 ml. of "hemoglobin solution." In these the second reading showed increases in the amounts of precipitate. In possible explanation of this it was noticed that the volume of the precipitated, centrifuged globulin in these tubes was about double that precipitated from those having only 0.1 ml. of "hemoglobin solution." The rest of the results, with specimens containing smaller amounts of "hemoglobin solution," so closely paralleled the control results that it seems likely the effect of hemoglobin on the globulin precipitation is probably small, within a rather wide range of concentration.

3. The ammonium sulfate solution.

It is recommended that "analytical reagent" grade of this salt be used, so that solutions used for globulin precipitation would be approximately the same regardless of the source of the chemical. In order to insure that the solution would be half saturated even at summer temperatures, it is made up with 48 Gm. added to each 100 ml. of distilled water. Five milliliters of this solution, plus 1 ml. of the serum from the hemolyzed specimen results in a solution that would be half saturated with ammonium sulfate at any temperature below 36° C.

SUMMARY

The problem of hemolysis in blood submitted to the St. Louis Municipal Bacteriological Laboratory is discussed. A method for performing a modified Kahn test on such "hemolyzed specimens" is described, and the results of comparative tests with this method and the standard Kahn test are given.

The modified Kahn test described herein for use with hemolyzed blood has been found to give approximately the same results as the standard Kahn test in comparative tests with a limited number of specimens. It is, therefore, being recorded with the view of suggesting comparative trial in laboratories which find hemolysis a definite problem.

I wish to express my gratitude to Dr. J. J. Bronfenbrenner and Mr. Nathan Nagle for advice and assistance in connection with the work.

REFERENCES

Evelyn, K. A., and Malloy, H. T.: Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood, J. Biol Chem. **126**: 655, 1938.
Kahn, R. L.: The Kahn Test—A Practical Guide, Baltimore, 1928, Williams & Wilkins Co.

A SIMPLE AUTOMATIC DISTILLING UNIT*

BRADFORD N. CRAVER, PH.D., AND HOWARD L. WILLIAMSON, B.ED*
DETROIT, MICH.

THE problem of redistilling two or three gallons of water every day can be troublesome when the largest flask available for a still has only the capacity of a liter. The use of a much larger flask is not satisfactory when only small burners are available for heating it. Accordingly, we devised the apparatus herein sketched to solve the problem. It permits continuous distillation throughout the day without, in our experience, requiring the slightest attention. A hasty survey of the literature plus consultation with our associates has failed to reveal a similar arrangement. Albeit, since the principles involved are so simple, they have undoubtedly been combined before to the same end. Hence, we make claim to nothing more than independent discovery. However, with the realization that other laboratories with limited apparatus may face a similar problem, we considered it worth while to make available a detailed description of the unit.

Essentially the siphon principle is combined with that of the Mariotte bottle. The bottom of the tube, *B*, in the Mariotte bottle, *A*, is adjusted so as to lie in the same horizontal plane as the surface of the water in the flask, *C*, when the flask has been filled to a level convenient for distillation. In practice the fluid usually rises somewhat higher in the flask *C* than the level calculated because the air in the Mariotte bottle *A* becomes progressively hotter as the distillation proceeds, due to the small amount of steam which bubbles back through the tube *D*. Accordingly, we usually adjust the Mariotte bottle, so that the end of the tube, *B*, is in the same horizontal plane as the surface of the liquid in the flask *C*, when the flask is about two-thirds full. The end of the siphon tube, *D*, is bent slightly upward and outward so that the orifice is near the wall of the flask. This tends to minimize the amount of steam which always bubbles back up through the tube, *D*, into the Mariotte bottle. Although this steam heats the water in the Mariotte bottles as the distillation progresses, in our experience this has neither interfered with the smooth operation of the unit nor cracked the nonpyrex Mariotte bottle. The lower opening of the tube, *D*, must, of course, be below the lower end of the tube, *B*, or the water will fail to siphon into flask *C*, as the distillation boils off the fluid in flask *C*. *H* is merely a plug of glass wool to prevent the entrainment of impure water droplets in the steam which passes over into the condenser, *E*. Any flask may serve as a receiver, *F*. *G* is a cotton plug to guard the opening of the receiver, *F*, so that no dust can fall into the receiver. The area of the condenser surface, if too small, will, of course, limit the rate at which one can distill since some of the steam, when too small a

*From the Department of Pharmacology, Wayne University College of Medicine, Detroit. Received for publication, April 22, 1942.

condenser is employed, will pass over into the receiver uncondensed. In actual operation the distillation is started with water in flask *C*, at the usual level, about two-thirds full. As the water in *C* boils down to a critical level about one inch above the bottom of the flask, water begins to siphon over into the still, *C*, from the Mariotte bottle, *A*. This cool water stops the boiling until flask *C* is again about two-thirds full, at which point siphoning ceases. With each succeeding filling the water will rise to a slightly higher level in flask *C*, because the air in the

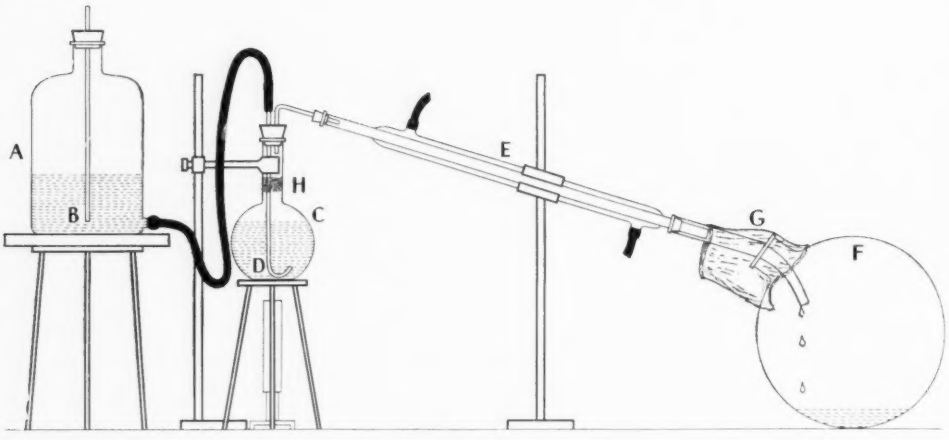


Fig. 1.

Mariotte bottle, as already noted, grows hotter as the distillation continues. All connections must be airtight and this precaution applies especially to the stopper in the top of the Mariotte bottle. With the precautions mentioned the distillation can be started early in the day and will smoothly proceed unattended as long as there is water in the Mariotte bottle. With our 10-liter Mariotte bottle it runs all day.

We offer this device in the hope it may prove of equal convenience to other laboratories faced with a similar problem. This arrangement is now being utilized by associates in other departments.

A METHOD FOR RAPID, REPEATED, APPROXIMATE DETERMINATIONS OF THE TRANSVERSE DIAMETER OF THE HEART

SAMUEL WALDMAN, M.D., BROOKLYN, N. Y.

THE routine determination of the transverse diameter of the heart is usually accomplished by means of teleoradiography, orthodiagraphy, and fluoroscopy.[1]

The teleoradiogram is the x-ray film of the heart, conventionally taken at a distance of six feet. This technique involves the use of x-ray equipment and x-ray film. In orthodiagraphy the examiner draws the outline of the heart in the visualized plane. This method requires much time and skill. With the fluoroscope, a direct examination is made, and contours can be studied in active motion.

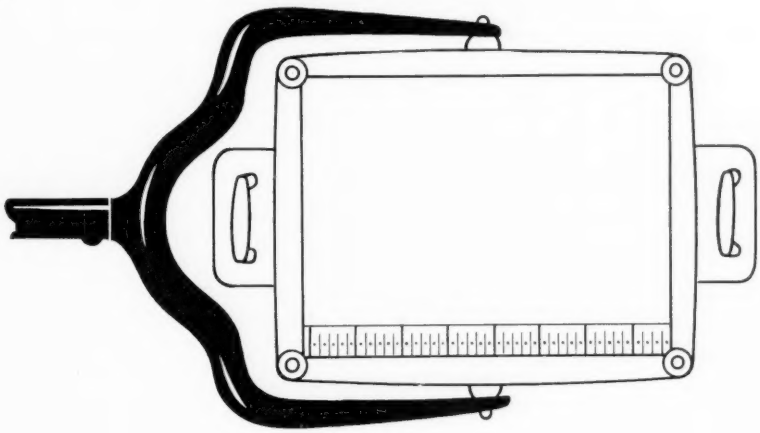


Fig. 1.

In order to determine rapidly and approximately the transverse diameter of the heart, I have devised a simple attachment to the fluoroscopic screen which, in a small degree, obviates the use of film and the orthodiagram, but which is not intended to supplant them.

For the conventional 12 by 16 inch fluoroscopic screen, a strip of x-ray film, ¾ by 16 inches, is cleared of emulsion by soaking in hot water. When it is thoroughly dry, lines are drawn on it with India ink as per diagram (Fig. 1). The width of the strip in the diagram is exaggerated for illustrative purposes. The ink is radiopaque and is easily seen during fluoroscopy of the chest. The complete lines indicate 5 cm., the incomplete lines 1 cm., and the dots 0.5 cm. Thus the accuracy is immediately limited to within 0.5 cm. The first line is drawn just within the edge of the screen. When ready, the strip is inserted into the base of the screen.

Received for publication, May 5, 1942.

By raising and lowering the screen, the widest diameter of the heart can be projected to this base line, and the measurement noted at once, giving the transverse diameter of the heart.

The patient must always stand touching his back to the fluoroscope, while the screen rests against the chest wall, and the head faces directly forward. The reading is taken at the end of inspiration. Under these conditions the measurements will be consistent and accurate. The figure obtained remains constant for the same patient under the same conditions. Repeated observations have borne this out. Little practice is required for rapid acquisition of the technique.

TABLE I

NAME	AGE	SEX	WEIGHT	HEIGHT (INCHES)	TRANSVERSE DIAMETER OF THE HEART		
					PREDICTED	OBTAINED	DEVIATION
Normal Hearts							
A. G.	27	M	145	67	125	130	5
H. W.	32	F	128	63	121	130	9
L. W.	24	F	130	60½	125	125	0
M. T.	33	F	157	62	135	130	5
A. S.	11	F	130	61	124	130	6
A. D.	29	F	108	62	112	120	8
L. W.	46	M	155	63	134	135	1
S. R.	26	F	136	63	125	130	5
S. D.	34	M	164	64	136	135	1
C. L.	24	F	101	59	110	110	0
J. G.	21	M	140	68	122	120	2
A. G.	19	M	171	69	134	135	1
E. W.	27	F	140	64	126	130	4
G. W.	24	F	169	68½	133	140	7
F. G.	43	F	137	62½	126	120	6
Hypertensive Hearts							
A. P.	49	F	181	62	146	165	19
F. K.	50	F	125	60	123	170	47
B. W.	61	M	145	64	128	160	32
M. R.	54	M	158	67	130	190	60
C. C.	51	M	146	64	128	142	14

This method of measurement has its greatest advantages when repeated studies of cardiac size are desired, especially at no material expense. Where changes in cardiac size occur, definite alteration in diameter can be determined at once. In the reversibly enlarged hearts of beriberi, myxedema, arteriovenous fistula, and cardiac dilatation, repeated measurements become invaluable and are easily determined by this method. Progressive enlargement or retrogression in the size of the heart can be observed easily, and recorded. Studies by Garland and McKenney[2] on the reversibly enlarged heart of thiamin deficiency revealed changes in the transverse diameter of the heart, as measured on 6 foot films, averaging a 2.7 cm. decrease in diameter in an average of fifteen days. The extremes varied from 1.0 to 4.5 cm. decrease in diameter of the heart, following the administration of thiamin. Such changes can be easily determined by the method herein described, without the use of film. Numerical records thus obviate somewhat repeated orthodiagrams and teleoradiograms.

Because of the closeness of the chest to the tube, the rays which strike the thoracic cage borders are too divergent for even approximate determinations of thoracic diameters which might conform to any tables. For the same reason

the larger the heart the more does its measure deviate from predicted figures. In this way, changes in size are more exaggerated than is actual, and therefore, more easily determined. The most accurate results are obtained in the normal heart size.

Ungerleider and Clark[3] in 1939 prepared tables of the transverse diameters of the heart, with which these measurements of the normal heart conform. The greatest deviation in the clinically normal heart was 9 mm. The least was 0 mm. Where clinical heart disease with enlargement existed, the figures roamed far from the predicted table.

In Table I are presented 15 patients with normal hearts, and five patients with hypertrophied hearts associated with hypertension. The comparative results by this method are easily seen.

SUMMARY

A simple method of immediate approximate measurement of the transverse diameter of the heart is described, obviating to a certain extent the use of orthodiagrams and teleoroentgenograms. Repeated studies in the same patient give excellent comparative results, and changes in the size of the heart are easily observed and recorded. Film is conserved, and time and expense are reduced to a minimum.

REFERENCES

1. Nomenclature and Criteria for Diagnosis of Diseases of the Heart, Criteria Committee of the New York Heart Association, New York, 1940, page 77.
2. Garland, L. H., and McKenney, A. C.: The Roentgen Diagnosis of Vitamin Deficiency Cardiac Conditions, Radiology **38**: 4, 1942.
3. Ungerleider, H. E., and Clark, C. P.: A Study of the Transverse Diameter of the Heart Silhouette With Prediction Table Based on the Teleoroentgenogram, Am. Heart J. **17**: 92, 1939.

1661 Prospect Place

APPARATUS AND TECHNIQUE FOR MEASUREMENT OF VIBRATORY THRESHOLD AND OF VIBRATORY "ADAPTATION" CURVE*

CHARLES D. ARING, M.D., CINCINNATI, OHIO, AND W. O. FROHRING, B.S., CHAGRIN FALLS, OHIO

IN THE clinical study of neurologic disorders attempts have been made to determine the appreciation of vibration. Little is known concerning the physiologic factors underlying this phenomenon other than the fact that the integrity of certain neural pathways is essential.

In order to elucidate the influence of various physiologic factors, such as circulation, respiration, emotion, and such physical factors as temperature and pressure on the appreciation of vibration, it was necessary to consider quantitative procedures. Numerous attempts at quantitative measurement had been made with but little headway until 1929, when an instrument was constructed for Tilney by Henney.[1] Subsequently, Laidlaw and Hamilton[2] improved this instrument (pallesthesiometer) and employed it to study the vibratory thresholds of various body surfaces. In spite of improvements, however, certain shortcomings still existed in the apparatus. It was our aim to devise an instrument which would retain the simplicity of the one constructed by Laidlaw and Hamilton, and at the same time eliminate certain variables which seemed inherent in their apparatus.

DESCRIPTION OF APPARATUS

The apparatus which was devised for these studies is illustrated in Fig. 1, the wiring diagram in Fig. 2. A Raytheon automatic voltage regulator was employed to reduce the voltage variation to plus or minus 0.5 per cent. The vibrating applicator has a standard resistance of 90 ohms and differs from the instruments employed by other investigators in that it vibrates horizontally, rather than vertically, to the surface of the area. Since an increase of pressure would tend to dampen the amplitude of vibrations applied vertically to the skin area, horizontal vibration was chosen, for it is less likely to be affected by pressure variation.

Results of preliminary tests on several subjects indicated that a maximum voltage of 50 would cover the most practical range with a 5,000 ohm potentiometer if the latter were "even" wound rather than "taper" wound. A dial divided into 100 divisions gives a more nearly logarithmic function of the amplitude than does a dial calibrated in arithmetic units. The arbitrary use of the dial reading for recording determinations seems to be entirely practical for comparing results. Because of possibility of error in calibration

*From the Neurological Service, Cincinnati General Hospital, the University of Cincinnati College of Medicine, and the Research Laboratories of the S. M. A. Corporation.
Received for publication, May 15, 1942.

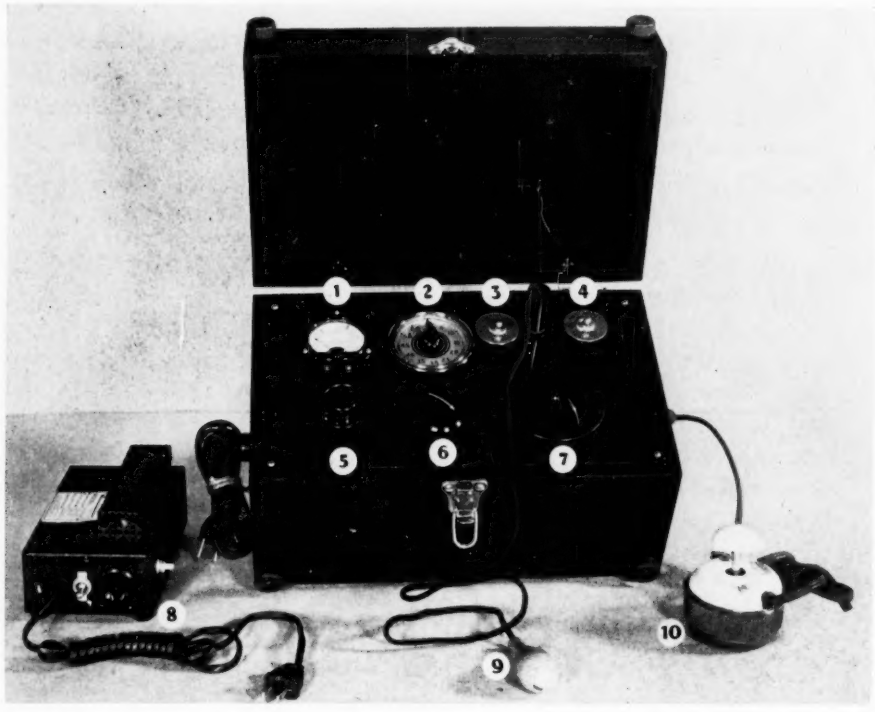


Fig. 1.—A portable pallesthesiometer.

1, Voltmeter.
2, Interval timer.
3, Switch for full amplitude of the vibrator.
4, Main switch.
5, and *6*, Voltage control knob.
7, Dial for recording amplitude of vibrator.
8, Electronic converter for use with direct current.
9, Push-button signal (operated by patient who indicates when vibration is felt).
10, Vibrator.

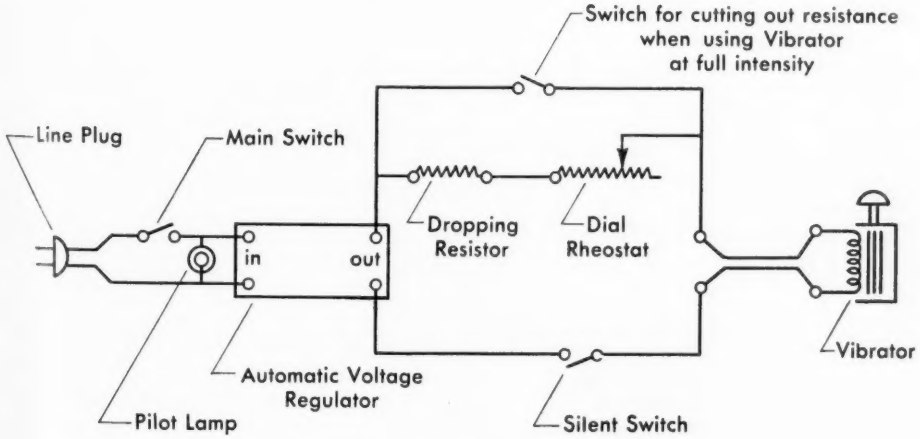


Fig. 2.—Wiring diagram of the pallesthesiometer.

methods, no attempt was made to calibrate the dial readings in terms of "units of energy" or "amplitude." In the preliminary studies, when the pallesthesiometer had an input of 110, the dial reading at zero (with the voltage across the vibrator) was 4½ volts; at a maximum dial reading of 93, 49 volts. In case the automatic voltage regulator is not used, a variable resistance must replace the dropping resistor in the vibrator circuit and a voltmeter must be placed across the leads to the vibrator. When only direct current is available, an electronic 60-cycle convertor may be inserted ahead of the instrument.

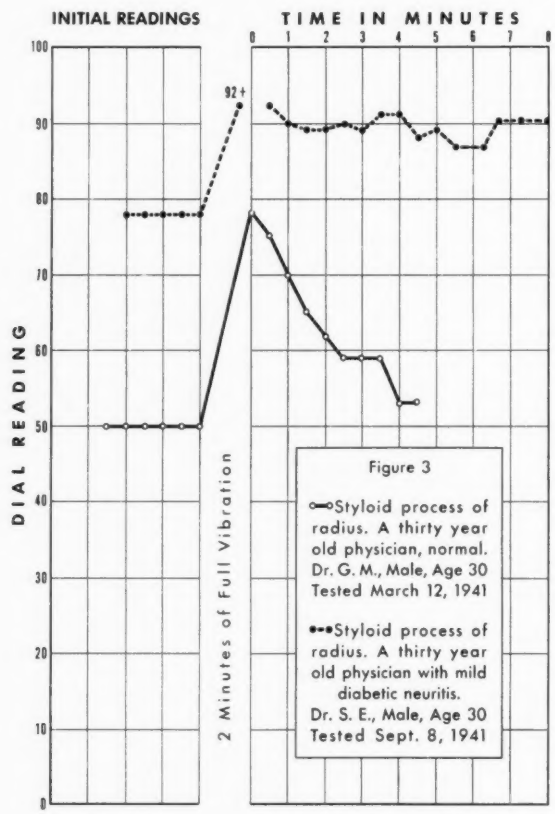


Fig. 3.—Vibratory "adaptation" curves.

METHOD

A modicum of attention is required on the part of the subject. After the procedure is explained, the subject may recline in the dorsal or ventral position. The vibrator is then applied to an area remote from that to be tested, so that the stimulus might be recognized before the test is begun. The applicator is held at right angles to the point tested, the weight of the entire applicator at that position representing roughly the pressure exerted on the skin. The initial threshold is then determined. With the dial set at zero and the vibrator applied to the test area, the dial is turned up slowly until the stimulus is perceptible. This process is repeated several times, allowing rest periods of thirty seconds to one minute between stimulations. It would be

logical to assume that threshold readings could be verified by starting with a stimulus heavier than the threshold, turning the dial toward zero, and recording the point at which the subject no longer perceives vibration. However, such a procedure results in an "adaptation" to the stimulus, particularly if the latter is heavy and is applied for more than an instant. Hence, it cannot be used to determine the threshold value.

The rate at which vibratory thresholds are approached has no effect on the final reading; that is to say, a variation in the speed at which readings are taken will not influence the subject's response. If he is reporting inaccurately, widely divergent readings are observed, indicating that the subject made his report when he thought the stimulus should have been evident rather than when it was actually felt.

The phenomenon of "adaptation" proved to be useful as a more delicate evaluation of vibratory appreciation than that educed by threshold determination alone. If, having determined the threshold of a surface over a given period of time, a heavy vibration was applied, the vibratory threshold was raised. When the "adapting" vibration was discontinued, it was found that the threshold returned to the original level in a period of a few minutes.

RESULTS

Vibratory thresholds were determined in normal persons. It was observed that readings in comparable test areas may vary widely from person to person. It is well known that the threshold varies from area to area on the body surface. Thus, Laidlaw and Hamilton[2] found that the vibratory threshold was lowest in the finger tips and highest at the sacrum.

Somewhat more constant was the adaptation to an intense stimulation. In the majority of subjects, return to the control threshold after a period of intense vibratory stimulation was observed in several minutes (Fig. 3). A much longer interval of return was observed in patients suffering from a variety of neurologic diseases (degeneration of the posterior columns of the spinal cord, subacute combined degeneration of the spinal cord, and peripheral neuritis) (Fig. 3). The influence of various physiologic and physical factors on vibratory appreciation in normal and abnormal states will be described in subsequent reports.

SUMMARY

An instrument for the quantitative measurement of the threshold of vibratory appreciation is described.

REFERENCES

1. Tilney, F.: Comparative Sensory Analysis of Helen Keller and Laura Bridgman; Its Bearing on Further Development of Human Brain, Arch. Neurol. & Psychiat. **21**: 1237, 1929.
2. Laidlaw, R. W., and Hamilton, M. A.: Thresholds of Vibratory Sensibility as Determined by the Pallesthesiometer, Bull. Neurol. Inst. New York **6**: 494, 1937.

A SIMPLE KIDNEY PLETHYSMOGRAPH*

FREDERICK E. EMERY, PH.D., BUFFALO, N. Y.

THE kidney can easily be approached, and by means of a little dissection can be freed from the abdominal wall and placed in a plethysmograph. Usually some difficulty is experienced in obtaining records of kidney volume because the plethysmographs adapted for the kidney have some disadvantages. *First*, they are difficult to make airtight; *second*, the vena cava or kidney vessels are often compressed, resulting in passive congestion; *third*, the instrument is frequently too large to be inclosed in the abdomen and, therefore, the temperature of the kidney may be below normal; and *fourth*, the composition of the plethysmograph prevents visual inspection of the inclosed tissue. With these points in mind a simple plethysmograph has been devised which eliminates all these objections.

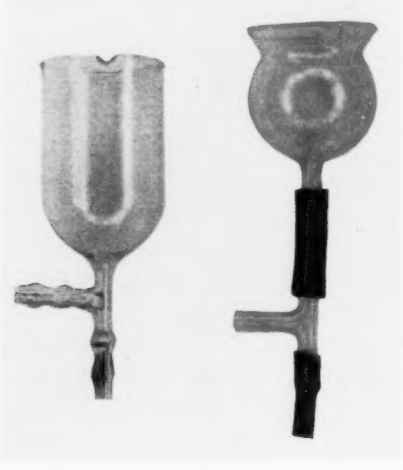


Fig. 1.

PLETHYSMOGRAPH

The plethysmograph is a cylinderical cup-shaped glass container. The bottom is entirely open and the top is closed, except for the opening of the outlet tube. The one shown on the right in Fig. 1 was made for large cats and is 40 mm. in diameter and 50 mm. high (this height is somewhat greater than actually needed). The two outlet tubes are clearly seen at the top of the picture, while at the bottom the notch intended for the passage of blood vessels is evident. This plethysmograph was made by a local glass blower. A workable, and in our experience equally satisfactory, apparatus for small cats can be made from a large thistle tube connected with a glass T-tube by means of rubber tubing (Fig. 1).

*From the Department of Physiology, University of Buffalo.

TECHNIQUE

The usual procedure of opening the abdomen and dissecting the kidney free from attachments is employed. The plethysmograph is prepared for use by placing a short rubber tube on the straight outlet arm. Through this tube, and extending through the bottom opening of the plethysmograph, a suture thread is passed. A small wire is used to pull the thread through the tube and plethysmograph. A suture needle is used to fix the lower end of this thread to the capsule of the kidney. The plethysmograph is now placed over the kidney, adjusted, and held in the desired position by means of a clamp from a base stand. The thread is pulled and the kidney is suspended in the glass chamber under as little tension as possible. A pinch clamp on the rubber

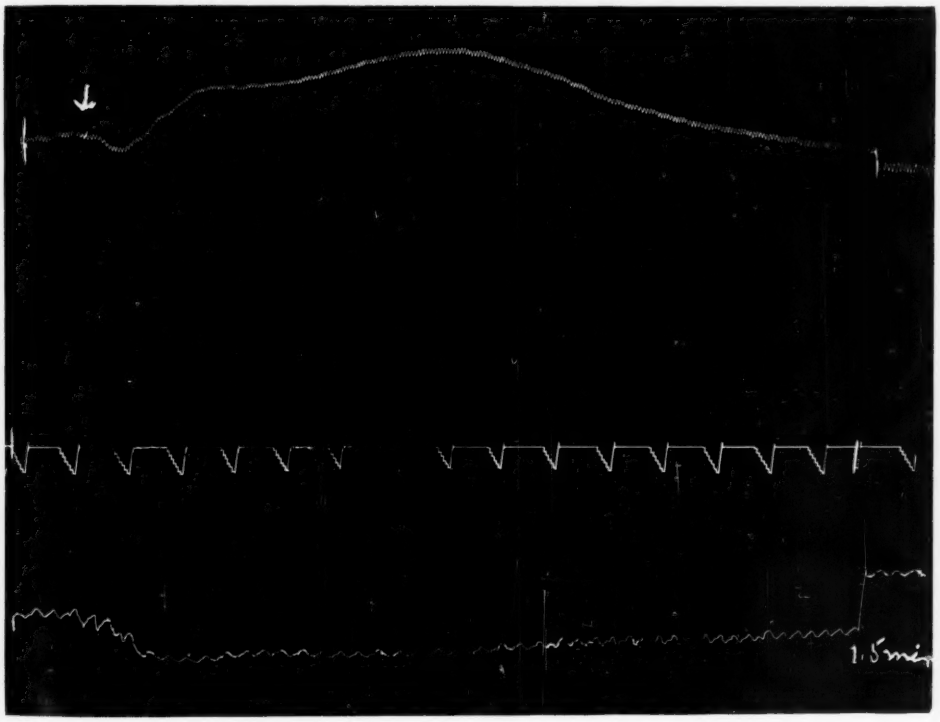


Fig. 2.—Blood pressure of a cat taken with a mercury manometer, upper tracing; base line and time in five seconds, middle tracing; and record of kidney volume, lower tracing. Note the return of the kidney volume to even less than normal during a rest period of one and one-half minutes. A single dose of 0.5 c.c. of a 1:50,000 solution of adrenalin given as indicated. Cat weight 2,000 Gm.

tubing holds the thread from slipping and closes the opening in the tubing. The kidney can be clearly seen and the plethysmograph can be adjusted so as to relieve all pressure on the vessels. A little warm mineral oil placed in the abdomen will make the plethysmograph airtight. Frequently no oil is required to make an airtight seal. If the intestines enter the plethysmograph, these may be blocked off with cotton rolled in vaseline, provided care is taken not to obstruct the veins of the kidney. The whole area is kept warm by closing the abdominal wall around and over most of the plethysmograph. The side arm of the plethysmograph is attached in the usual way to the recording tambour.

Records taken of kidney volume usually clearly show respirations and heartbeats, as well as changes in volume due to vasomotor activity. Fig. 2 shows the effect of adrenalin injected intravenously in an anesthetized cat. The expected rise of blood pressure occurs and constriction of the kidney, as indicated by the fall in the plethysmographic record, is evident.

AN ACCURATE CONSTANT TEMPERATURE WATER BATH CONSTRUCTED AT VERY LOW COST*

BENJAMIN C. RIGGS, M.D., PHILADELPHIA, PA.

THE need for a small, accurate, constant temperature water bath of low cost led to the development of the design described. The accuracy was to be within 0.1° C. and the capacity 2 liters. The final cost came to less than four dollars. The uses for such a bath in the office or hospital laboratory are readily apparent and include blood amylase and lipase, trypsin, urea, amino acids, calcium, gastric enymes, inactivation of serum, and vaccine preparation.

Theory.—Essentially, the apparatus consists of an electrically powered immersion heater, controlled by a simple thermoregulator, installed in a small water bath of suitable shape to insure thorough mixing by convection.

The relation between vapor tension and temperature has long been known and practically used. In the design described, a mercury column, driven upward by the rising vapor tension of a suitable fluid subjected to rising temperature, is coupled at an adjustable point to a small mercury manometer. One arm of this manometer serves as the moving element of a single pole switch, making and breaking the power circuit of the heater. Undesirable arcing in the switch is eliminated by a low capacity condenser connected across the switch circuit.

The heating element also uses a well-known principle. The resistance to alternating current of a low concentration of electrolytes in water is used to produce high temperatures within a small space. In this design, two heavy gauge, parallel wires are immersed in the tap water of the bath and connected to either side of the 110 volt A.C. house circuit through a thermoregulator. When this is in closed position, the water between the exposed wires is kept at boiling temperature. Power consumption is regulated to suit the size and temperature of the bath by a sliding insulator covering one of the wires.

To eliminate the conventional mechanical stirrer, the heater is placed centrally in an approximately hemispherical bath, with the result that convection currents eliminate any temperature variations outside the sensitivity limits of the thermoregulator. (The presence in the bath of test tubes, flasks, etc., presents no measurable interference if the spacing between each is at least 2 cm.)

Construction.—1. Heater. (Cost of materials, approximately 50 cents.)

For temperatures up to 45° C., a single pair of parallel, 18 gauge wires, of 6 cm. exposed length, 2 mm. apart, will deliver sufficient heat to maintain

*From the Laboratory of the Germantown Hospital, Philadelphia.
Received for publication, May 19, 1942.

the temperature in 2 liters of water; the circuit will be closed not more than 50 per cent of the time. For higher temperatures or larger baths a double unit is more satisfactory: two pairs of wires are used, one pair operating continuously; the other, connected in parallel, running intermittently through the thermoregulator circuit. The construction is essentially the same in either case.

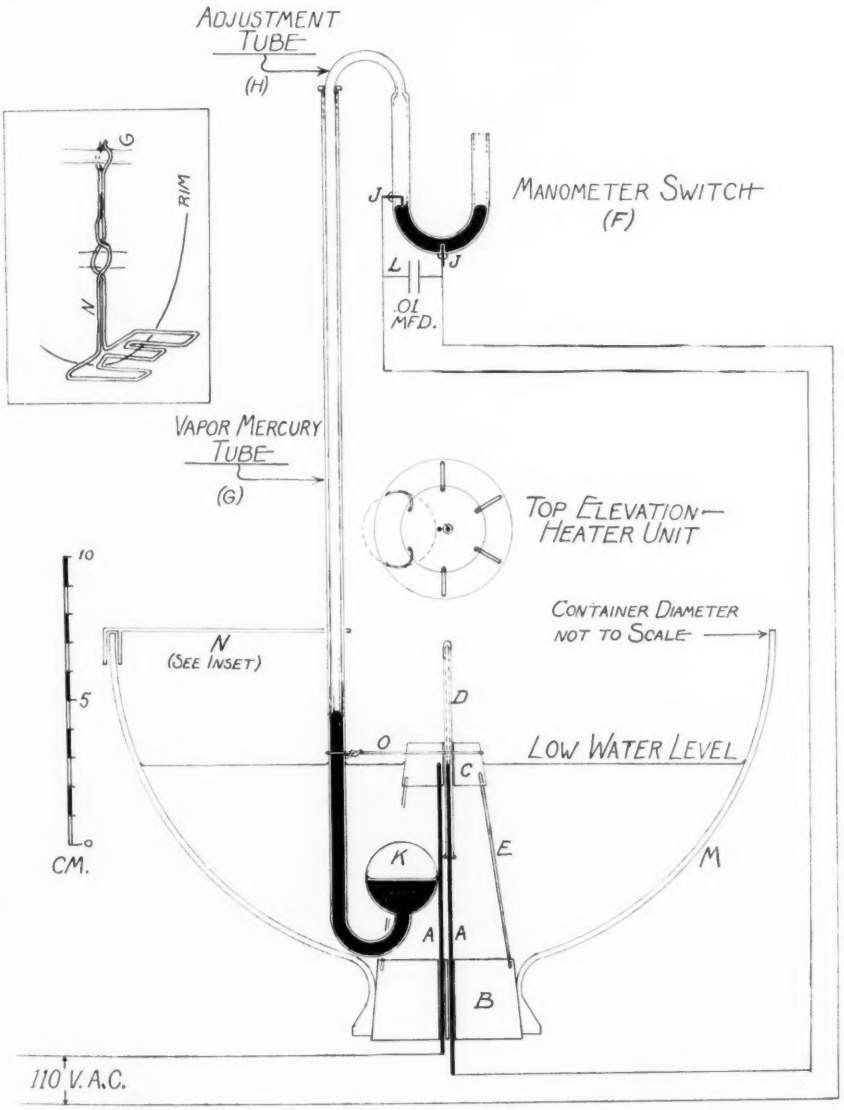


Fig. 1.

Although the apparatus now in use was built with copper heating wires, it is probable that some other metal, such as stainless steel or silver, would be less liable to the formation of objectionable salt crusts.

The heating wires (*A*) are passed through the rubber stopper (*B*) forming the base (of a size to fit tightly the neck of the container), and are secured by insertion into the rubber stopper (*C*) forming the top of the heater. A single

hole is bored in (*C*), at the insertion of one wire of each pair, to fit the glass insulating tubes, shown (*D*) in the single unit. These are lubricated with stopcock grease; their position regulates the amount of exposed wire surface, and hence, the power consumption. A guard is built around the heating wires of painted iron wires (*E*), six in number, spaced evenly about the periphery, the ends inserted in the top and bottom stoppers. Two of these will be spread slightly to accommodate the vapor bulb (*K*) after it is constructed.

2. Thermoregulator. (Cost of materials approximately $2.50.)

Two sizes of soda lime glass tubing are used and enough "881" glass to form two beads about 2 mm. in diameter. The switch (*F*) and vapor mercury tube (*G*) have an *inside* diameter of 5 mm., and the adjusting tube (*H*) has an *outside* diameter of 4 mm. The "881" glass is used as a sealing medium around the platinum switch electrodes (*J*), because its coefficient of expansion is almost exactly that of platinum, thereby avoiding cracks. The vapor bulb (*K*) is blown to approximately 2.5 cm. diameter from the sealed end of the vapor mercury tube (*G*) *before* bending. Properly placed holes, 1 mm. in diameter, are blown in a *previously* bent section of the same sized tubing to form the manometer switch (*F*). One centimeter lengths of 20 gauge platinum wire (*J*) are sealed into these holes in beads of "881" glass. The adjusting tube (*H*) is sealed to the manometer *after* bending, and is fitted with a snug rubber washer cut from tubing to enable it to be set at any desired height within the vapor mercury tube (*G*). The 0.01 microfarad tubular condenser (*L*) is mounted between the outside ends of the electrodes to facilitate wiring and give support to the electrodes and seals. The mercury in the switch should just make thorough contact with the upper electrode, with both sides unobstructed, and the adjusting tube vertical.

The vaporizing fluid in the vapor bulb is best chosen by trial and error for the temperature range contemplated. Satisfactory results have been obtained with mixtures of ethyl ether and absolute ethyl alcohol, for temperatures from 35° C. to 45° C., and with petroleum ether from 40° C. to 60° C. The amount of fluid, size of air bubble, and amount of mercury are also best found by experiment. (The author used mercury 10 c.c. and fluid 0.5 c.c.)

The wiring should be evident from the diagram. A second heating unit is simply wired directly across the power lines. It has been found convenient to wire the manometer switch to a plug, fitting into a socket at the base of the heater, to facilitate removal for cleaning the bath.

3. Container (*M*). (Cost of materials, approximately $1.00.)

The most satisfactory container was found to be an inverted glass lamp shade of hemispherical shape, the neck receiving a No. 11 rubber stopper, the rim about 30 cm. in diameter. This pattern is easily obtainable for about $1.00 and has been perfectly suited.

The thermoregulator is so placed that the vapor bulb passes between two of the guard wires, slightly spread to receive it, and rests in contact with the heating element. (This should be the intermittent heating element if a double unit is used.) The vapor mercury tube is held vertically by a simple clamp (*N*) bent from iron wire, slipping onto the rim of the container. An auxiliary clamp

(*O*) is bent in the form of a double-ended hook to steady the bulb against the heating element. Clamp (*N*) is also bent to receive a standard, mercury immersion thermometer.

Operation.—The apparatus is designed for use on the standard, 110 volt A.C. circuit. Continuous operation requires a daily check on the water level and flushing out of the bath weekly. No other attention has been found necessary. When beginning use, the bath should be filled with water at approximately the desired temperature, and the adjusting tube raised so that the switch just makes contact. Final adjustment is postponed until the thermoregulator and bath have reached equilibrium, or about one-half hour after filling. The maximum over-all variations in temperature have not been observed to exceed 0.2° C.

SUMMARY

A new design is described for an extremely inexpensive, small, easily constructed, constant temperature water bath, for use in the hospital or office laboratory. The theory, construction, and operation are given in detail.

LEVINSON TEST FOR TUBERCULOUS MENINGITIS*

Presentation of 110 Cases and a Discussion of the Principle and Technique of the Test

Daniel Burman, A.M., M.D., and Roslyn Weintraub, B.A.
New York, N. Y.

INTRODUCTION

THE Levinson test for tuberculous meningitis is a very accurate diagnostic procedure. A study of the literature and a personal survey of several hospitals and many physicians revealed that this test is little known and used still less. As a result, we feel that a presentation of the results of this test on 110 cases, a review of the results of previous investigations, and a discussion of the principle and technique will be of value and may serve to increase the use of this valuable adjunct in the diagnosis of meningitis.

HISTORY

In 1917 Tashiro and Levinson[1] published a report on the alkaloidal and metallic precipitation of cerebrospinal fluid in the diagnosis of meningitis. It was not until 1919 that this test again appeared in the literature. At that time Levinson[2] included it in his book on spinal fluid without discussing its principle. The first clinical report that we could find on this subject was the paper published by Gleisch[3] in 1932. In this paper he discusses the test and reports that in his cases the results were uniformly good. The same author[4] again published a clinical report in 1938 in which he states that 51 cases (Tables I, II,

*From the Otolaryngology and Pathology Departments of Harlem Hospital, New York, N. Y.

Received for publication, May 22, 1942.

III) were examined and tested by this method with very good results. The report showed 38 cases with a positive Levinson test and 13 cases with a negative result. Of the 38 cases, 23 revealed tuberculosis organisms; 6 showed tuberculosis at autopsy; one tuberculous patient had tuberculosis organisms in the spinal fluid; and 8 did not have tuberculosis. However, of these 8, one had an intracranial hemorrhage with multiple lacerations; 2 had cerebral hemorrhage with hypertensive cardiovascular disease; one had encephalitis lethargica; 3 were epileptics; and one had staphylococcus meningitis. All the 8 nontuberculous meningitis cases were positive, since there was either blood or serum present in the spinal fluid. Of the 13 negative tests all were nontuberculous, 10 had a meningococcic meningitis, and 3 had poliomyelitis.

TABLE I

	Positive—38	Negative—13
Tuberculous	30	0
Nontuberculous	8	13

TABLE II

Nontuberculous positives	8
Intracranial hemorrhage	3
Encephalitis lethargica	1
Epilepsy	3
Staphylococcus meningitis	1

TABLE III

Nontuberculous meningitis	13
Meningococcic	10
Poliomyelitis	3

In 1939 Gleisch and R. Weintraub[5] published a study of the comparative value of the Levinson and tryptophan tests in the diagnosis of tuberculous meningitis. They pointed out that the tryptophan test was markedly inferior to the Levinson test. Their results showed (Table IV) that in 18 cases of positive tuberculosis all had positive Levinson tests, but only 16 had a positive tryptophan A, 12 a positive tryptophan B, and 2 were negative in both tryptophan A and B tests. Furthermore, in 8 cases of nontuberculous meningitis with clear fluid (Table V) all had negative Levinson tests, but 8 had a positive tryptophan A and 3 had a positive tryptophan B. They also showed that in 7 cases of bloody spinal fluid and 9 cases of purulent meningitis (Table VI) all had positive Levinson and tryptophan tests; and that in 14 cases without meningitis all had negative tryptophan and Levinson tests.

In 1940 S. Weintraub[6] published a paper on the various diagnostic procedures for tuberculous meningitis. He was favorably impressed with the results of the Levinson test.

TECHNIQUE

Into each of two small test tubes of uniform width, about 0.3 cm. in diameter, place 1 c.c. of spinal fluid. Add to one of the tubes 1 c.c. of 3 per cent sulfosalicylic acid and to the other 1 c.c. of 1 per cent mercuric chloride. Stopper the tubes and allow them to stand for from twenty-four to forty-eight hours at room temperature and then measure the height of the sediments. (The very small flat-bottomed serum vials are very useful tubes to use for this test.) Normally, both are very small; in suppurative meningitis the sulfosalicylic precipitate is very heavy and the mercuric chloride precipitate is very small. In

tuberculous meningitis the opposite takes place, the sulfosalicylic acid precipitate being small and the mercuric chloride precipitate being large. In order to read a positive Levinson test the foregoing conditions must be fulfilled and the mercuric chloride precipitate must be from two to three times as great as the sulfosalicylic precipitate; that is, when measuring the height of the precipitate in the two tubes, diameters of which are the same, the height of the mercuric chloride precipitate must be two to three times as large as that of the sulfosalicylic acid. It is to be remembered that this test is only to be performed on clear spinal fluid.

TABLE IV

COMPARATIVE VALUE OF THE LEVINSON AND TRYPTOPHAN TESTS IN 18 CASES OF PROVED TUBERCULOUS MENINGITIS

	LEVINSON	TRYPTOPHAN A	TRYPTOPHAN B	TRYPTOPHAN A AND B
Positive	18	16	12	12
Negative	0	2	6	2

TABLE V

EIGHT CASES OF NONTUBERCULOUS MENINGITIS WITH CLEAR FLUID

	LEVINSON	TRYPTOPHAN A	TRYPTOPHAN B
Positive	0	8	3
Negative	8	0	5

TABLE VI

SIXTEEN CASES WITH BLOOD OR PUS IN THE SPINAL FLUID (BLOOD 7—PUS 9)

	LEVINSON	TRYPTOPHAN
Positive	16	16
Negative	0	0

FOURTEEN CASES WITH NONMENINGITIC SPINAL FLUID

	LEVINSON	TRYPTOPHAN
Positive	0	0
Negative	14	14

PRINCIPLE

The spinal fluid of tuberculous meningitis, like normal spinal fluid, has a pH of approximately 7.4 immediately after withdrawal, but ascends to 8.1 or higher on standing for from twenty-four to forty-eight hours. Spinal fluid from epidemic meningitis has a pH 7.2 to 7.5 but goes up only slightly on standing from twenty-four to forty-eight hours. The more turbid the fluid the longer it retains its acidity. An explanation that has been offered for this phenomenon is as follows: Normal spinal fluid loses H ions on standing. However, if it is tightly corked it does not change its pH. Thus, this phenomenon may be due to the loss of carbon dioxide. Tuberculous meningitic spinal fluid reacts just as the normal spinal fluid, only more rapidly. Epidemic meningitic spinal fluid changes its pH very little and becomes more acid on being corked. This may be due to lactic acid formation.

Bacteriology is helpful in making a diagnosis when the results are positive. However, it is not of much aid when negative results are obtained. Globulin is increased in all meningeal inflammations, but the amount of increase does not help to determine the disease because the ranges overlap each other.

The pH of spinal fluid changes on standing and varies between epidemic and tuberculous meningitis, as discussed above. Levinson stated in 1917: "If the difference in the reaction of the different fluids is great enough, we should

be able to distinguish their protein in a variety of ways, even if their protein concentration might be the same. The electrical charge of a protein depends on the reaction of the medium. In acid solution proteins become electro-positive and in alkaline solution electro-negative. Thus one of the ways of precipitating protein is to let it combine with some radical to form an insoluble salt. From the standpoint of the electrical charge of the protein, such a protein salt is necessarily either a positive protein radical forming a protein salt with the negatively charged ions (alkaline precipitates) such as tungstic acid, picric acid, etc., or negatively charged protein combining with a positively charged metal (metallic precipitates) such as copper, silver, mercury, zinc, and lead. If these protein salts are sufficiently insoluble, the precipitate will come down.''

TABLE VII

ONE HUNDRED AND TEN CASES EXAMINED BY LEVINSON TEST

Proved tuberculous meningitis		68
Positive tests	64	
Negative tests	2	
Equivocal tests	2	
Controls		33
Meningismus		
Nontuberculous meningitis		
Nonmeningitic		
Discarded cases		9
Red blood cells	6	
Quantity insufficient	3	

TABLE VIII

SIXTY-EIGHT CASES OF PROVED TUBERCULOUS MENINGITIS

Diagnosed by finding the organism in the spinal fluid or on autopsy	41
Diagnosed by clinical, x-ray, and other laboratory tests	27
Deceased	68

Cataphoresis experiments proved that the protein in epidemic meningitis moves toward the cathode, showing the presence of positively charged protein ions, whereas in tuberculous meningitis the protein ions went to the anode, proving a greater number of negatively charged protein ions in this condition. In nonmeningitic fluids there is very little precipitation in cataphoresis. Thus the pH variation is enough to produce electrical charges in the protein. Therefore, it should be possible to choose proper precipitants to distinguish the various forms of meningitis; that is, there should be more precipitate with a metallic precipitant than with an alkaloid precipitant in tuberculous meningitis, and the reverse in epidemic meningitis. Experimental work subsequently proved this to be true.

Normal fluid is only slightly turbid when sulfosalicylic acid is added and remains clear with mercuric chloride. This condition obtains only immediately after the addition of these reagents. However, on standing both form precipitates. In tuberculous meningitis the addition of sulfosalicylic acid causes the fluid to become turbid immediately whereas with the addition of mercuric chloride the fluid remains clear. In twenty-four hours sediments are found in both tubes, but the precipitate due to the mercury is two to three times as much as that due to the sulfosalicylic acid. In epidemic meningitis the sulfosalicylic acid precipitates the protein quickly and the mercuric chloride precipitates the protein slowly. In this case twenty-four hours after the addition of the pre-

cipitants the sulfosalicylic acid sediment is two to three times as great as that of the mercuric chloride. It cannot be overemphasized that the principal feature in this test is the ratio and not the total amount of precipitate which is found, and this is directly dependent upon the pH of the protein.

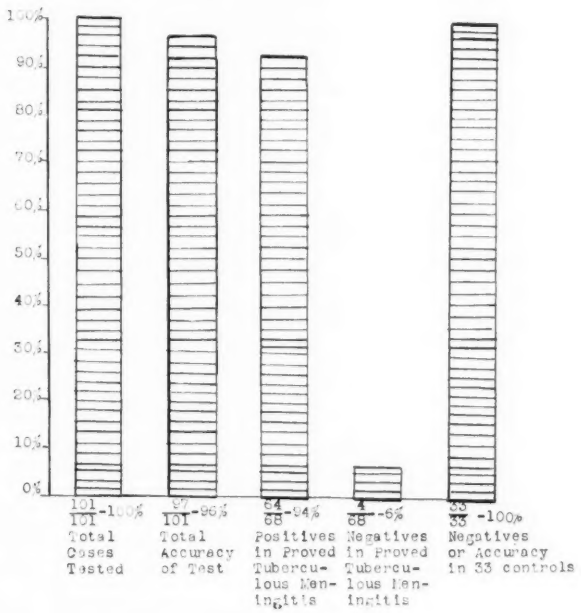


Fig. 1.

CASE STUDIES

The spinal fluids of 110 cases were examined. Nine of these had to be discarded for the following reasons: Six had red blood cells and three had insufficient fluid for a Levinson test after the other tests had been performed. As a result we have only 101 cases on which tabulations can be based. There were 68 cases of tuberculous meningitis and 33 controls. These 33 cases were diagnosed as meningismus, nontuberculous meningitis, or cases in which no meningeal signs were present. Sixty-four of the 68 cases were positive while 2 were negative and 2 gave equivocal results when tested by the Levinson method; that is, 2 could have been read either way—positive or negative. We are considering these as negative results. Thus we have 4 negative results. Of the 33 controls all gave negative results in this test.

Forty-one of the 68 cases, or 60.3 per cent, were proved to be tuberculous meningitis by one or more of the following methods: finding the organism in the spinal fluid or autopsy. The other 27, or 29.7 per cent, were diagnosed as tuberculous meningitis on the basis of clinical, x-ray, and other laboratory findings plus the fact that they all died. Thus 64/68, or 94 per cent, gave positive Levinson tests, and 4/68, or 6 per cent, gave negative Levinson tests. Furthermore, all the controls, that is, 33/33, or 100 per cent, gave negative Levinson tests; or, to express it in another way, 97/101, or 96 per cent, gave accurate results by the Levinson method.

If we now combine our results with those reported above, taken from the literature, there is a total of 217 cases. Of these, 116 were proved to be tubercu-

lous meningitis and gave 112 positive results and only 4 negatives. One hundred and three were nontuberculous and gave the following results:

Positive Levinson tests 30

All had either red blood cells or pus in the spinal fluid

Negative Levinson tests 70

These were made up of normals and cases of poliomyelitis, meningismus, nontuberculous meningitis, and 2 equivocal cases.

Quantity Insufficient for a Levinson test 3

After all the routine tests were performed, there was not sufficient spinal fluid left for the performance of the Levinson test.

COMMENT

We have presented 217 cases which were studied for the determination of the accuracy of the Levinson test. Of these, 110 were our own and 107 were from the literature. Our results revealed that this test is 94 per cent accurate, whereas the total of our cases plus those of the other investigators (who found 100 per cent accurate results) brings the percentage of accuracy up to 97. As this compares favorably with any other diagnostic laboratory procedure, and as the number of cases studied is not too small, we feel that this simple laboratory test should be used more often in the diagnosis of meningitis.

It should be evident from this paper that the test is of value only in cases of meningitis where the spinal fluid is clear and where no serum has been administered, since pus, red blood cells, and serum render the spinal fluid more alkaline and result in a false positive.

SUMMARY

1. The history and a summary of the literature on the Levinson test are presented.

2. A description of the technique and the principle on which the test is based are given.

3. A total of 110 new cases and 107 cases in the literature on which the Levinson test was performed, together with a breakdown of the results to determine the accuracy of the test, is presented.

4. Tables showing the test from 94 to 97 per cent accurate in diagnosing tuberculous meningitis are included.

5. A bibliography of all the available literature on this subject is presented.

REFERENCES

1. Tashiro, Shiro, and Levinson, A.: Alkaloidal and Metallic Precipitation of Cerebrospinal Fluid in the Diagnosis of Meningitis, J. Infect. Dis. **21**: 571, 1917.
2. Levinson, Abraham: Cerebrospinal Fluid in Health and Disease, 1919, page 193.
3. Gleisch, Morris: Levinson Test in Tuberculosis Meningitis, Am. J. Dis. Child. **43**: 1077, 1932.
4. Gleisch, Morris: Levinson Test in Tuberculosis Meningitis, Am. Rev. Tuberc. **38**: 239, 1938.
5. Gleisch, Morris, and Weintraub, Roslyn: Comparative Value of the Levinson Test, Arch. Pediat. **56**: 749, 1939.
6. Weintraub, Solomon: Laboratory Procedures in Tuberculous Meningitis, Bull. Am. Acad. Tuberc. Physicians No. 5, pages 13-18, February, 1941.

667 MADISON AVENUE
NEW YORK, N. Y.

CHEMICAL

THE DETERMINATION OF TOTAL CHOLESTEROL AND CHOLESTEROL ESTERS IN BLOOD*

S. L. Leiboff, New York, N. Y.

METHODS for the quantitative determination of cholesterol esters in blood have been devised by Bloor and Knudsen,[1] Sperry,[2] Kelsey,[3] and others. In all these methods the free cholesterol is separated from the esters by precipitation with digitonin. For the quantitative estimation of both free and combined cholesterol, practically all methods utilize the Liebermann-Burchard color reaction.

Various procedures are used for extracting the cholesterol from blood. Bloor[4] extracts it from wet blood with a mixture of alcohol and ether. Myers and Wardell[5] dry blood with plaster of Paris and extract with chloroform. I[6] dry the blood on fat-free filter paper and extracts with chloroform.

Because of the difficulties inherent in these methods, requiring a great deal of time and painstaking manipulation, a study was undertaken to determine the conditions best suited to produce the quickest and most accurate results. This study culminated in the development of an extremely simple and accurate method for the determination of total cholesterol and cholesterol ester content in blood.

PRINCIPLE OF METHOD

Oxalated blood is mixed well with anhydrous sodium sulfate and extracted with chloroform for twenty minutes in a graduated test tube attached to an air-cooled condenser. The chloroform extract is filtered and two aliquot portions are removed. One portion is treated directly with acetic anhydride and sulfuric acid for the total cholesterol content. The second portion is used for the determination of esters as follows: The chloroform is removed by evaporation and substituted by petroleum ether from which the free cholesterol is removed with digitonin. The petroleum ether is evaporated, the esters are taken up in chloroform, and the color is developed with acetic anhydride and sulfuric acid. Both fractions are determined colorimetrically against a standard containing a known amount of cholesterol.

Preparation of Reagents.—It is important that the reagents used in the test be of the highest purity; this applies particularly to the chloroform and acetic anhydride. The following methods for the purification of these reagents have proved very satisfactory.

Purification of Chloroform.—Into a 2,000 c.c. separatory funnel introduce 1,000 c.c. of chloroform and 100 c.c. of concentrated sulfuric acid. Shake at intervals during twenty-four hours. Remove the sulfuric acid and wash the

*From the Laboratory of Lebanon Hospital, New York.
Received for publication, March 15, 1941.

chloroform with a slow stream of running water passing through a glass tube reaching to the bottom of the separatory funnel until neutral to litmus. Transfer the chloroform to a distilling flask and add 50 Gm. of anhydrous calcium chloride. Shake at intervals during twenty-four hours and distil, discarding the first and last 50 c.c. of chloroform. Transfer the distilled chloroform to an amber bottle and add 0.7 c.c. of absolute alcohol per 100 c.c. of chloroform and a few grams of anhydrous calcium chloride. The alcohol acts as a preservative. Keep the bottle tightly stoppered.

Purification of Acetic Anhydride.—Heat 500 c.c. of acetic anhydride with 50 Gm. of calcium oxide in a distilling flask under a reflux condenser for three hours. Allow to stand overnight and distil slowly. Keep in brown bottle with tightly fitting glass stopper.

Preparation of Digitonin Reagent.—In a small flask place 1 Gm. of digitonin, 10 c.c. of methyl alcohol, and 10 c.c. of distilled water. Place the flask in a beaker containing water heated to about 60° C. and rotate the flask until the digitonin is completely dissolved.

On standing the digitonin separates out of solution. It is, therefore, necessary to redissolve it by placing in hot water before use. It redissolves very readily.

Preparation of Cholesterol Standard.—Prepare a stock solution by dissolving 0.160 Gm. of pure cholesterol in 100 c.c. of chloroform. A working standard is prepared by diluting 5 c.c. of the stock standard with 95 c.c. of chloroform. Five cubic centimeters of the working standard contain 0.4 mg. of cholesterol. The standards are kept in the refrigerator in amber bottles.

Extraction Apparatus (Fig. 1).—The apparatus consists of a graduated glass tube 20 by 150 mm. The graduation marks start at zero about 40 mm. from the bottom of the tube and continue upward to a mark of 60. These graduation marks do not represent specific volumes but are used to indicate the chloroform level in the tube. The inside mouth of the tube is ground to fit a coiled glass air-cooled condenser. The whole length of the condenser is 260 mm. and the coiled portion consisting of six coils is 100 mm. long and 10 mm. wide. This condenser is a modification of the one suggested by Breh.[7]

The glass rod is 220 mm. long and 5 mm. in diameter.

PROCEDURE

Determination of Total Cholesterol.—When a determination of the total cholesterol only is required, the following procedure is to be followed:

Place 0.5 c.c. of oxalated blood into the extraction tube and add approximately 2 Gm. of anhydrous sodium sulfate and exactly 10 c.c. of chloroform. Mix with a glass rod to a homogeneous mixture by pressing and rubbing the rod against the walls of the tube. Hold up the extraction tube in a perfectly vertical position level with your eye, and without removing the rod, read the graduation mark at the meniscus of the chloroform. Attach the condenser to the tube without removing the rod, and heat gently over a microburner or an electric heater for ten minutes. Allow to cool for a few minutes, detach the condenser, and stir up the powder thoroughly with the rod. Attach the condenser and heat for ten more minutes to complete the extraction. Allow to cool to room temperature and add chloroform to the original graduation mark. Mix well with

the rod and filter through a very small piece of absorbent cotton packed somewhat tightly in the stem of a small glass funnel. Keep the funnel covered with a watch glass to prevent evaporation.

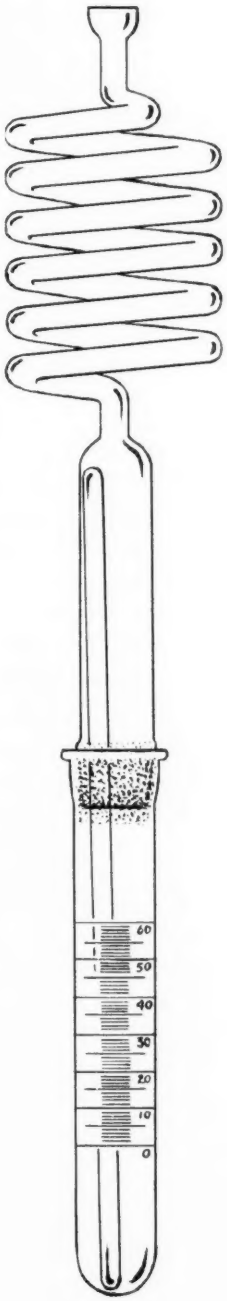


Fig. 1.

Transfer 5 c.c. of the chloroform extract to a test tube. In another test tube place 5 c.c. of the working standard (equal to 0.4 mg. cholesterol). Place the standard and the unknown in a beaker of water kept between 20° and 25° C.

Add to each tube 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid. Cover the tubes with clean dry corks, mix contents, and set aside in a dark place for twenty minutes. Compare colors in the colorimeter by setting the standard at 15 mm.

Calculation of Result.—

$$\frac{2400}{X} = \text{Milligram of cholesterol per 100 c.c. of blood.}$$

$$X = \text{Reading of the unknown.}$$

Determination of Total Cholesterol and Cholesterol Esters.—Place 1.5 c.c. of oxalated blood into the extraction tube and add approximately 6 Gm. of anhydrous sodium sulfate and exactly 15 c.c. of chloroform. Mix well with the rod and note the graduation mark at the meniscus of the chloroform. Extract for twenty minutes and filter through absorbent cotton, following all the steps already described under the determination of total cholesterol.

Transfer 2.5 c.c. of the chloroform to a test tube and add 2.5 c.c. of chloroform. Stopper with a clean cork and set aside for the determination of total cholesterol.

Transfer 7.5 c.c. of the chloroform extract into a test tube (20 × 150 mm.). This is for the determination of cholesterol esters. Place this tube in a water bath heated to about 70° C. until all the chloroform is driven off. Cool to room temperature and add 9 c.c. of petroleum ether (B. P. 30° to 60° C.) and 0.1 c.c. of digitonin solution previously heated to put in solution. Cork the tube and shake well; the cholesterin digitonide is precipitated immediately. Allow to stand for a few minutes and filter through a small piece of cotton tightly packed in the stem of a small funnel. In this case the cotton has to be packed very tightly so that no particles of digitonide can pass through.

Remove 6 c.c. of the petroleum ether filtrate into a similar large test tube and place in the water bath at about 60° C. until all the petroleum ether is completely evaporated. Allow the tube to cool to room temperature and add 5 c.c. of chloroform. This contains the cholesterol esters only.

Into a third tube place 5 c.c. of the cholesterol working standard.

Place the two unknowns and the standard in a beaker of cold water at a temperature between 20° and 25° C., and to each tube add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid. Mix the contents and put the beaker in a dark place for twenty minutes and compare in the colorimeter with the standard set at 15 mm.

Calculation of Results.—

Total Cholesterol: 2,400 divided by reading of unknown
= milligrams per 100 c.c. of blood.

Cholesterol Esters: 1,200 divided by reading of unknown
= milligrams per 100 c.c. of blood.

Precautions.—The color developed by the action of acetic anhydride and sulfuric acid on cholesterol changes rapidly on removal from the water bath and on exposure to light. Since this change is at a different rate in the standard and in the blood extracts, it is important that the colorimetric matchings be made

as rapidly as possible after removal from the dark. It should not exceed three minutes. When a large number of cholesterol determinations are to be performed simultaneously, it is advisable to set up a number of standards and use a new standard every three minutes. Each standard is removed from the beaker simultaneously with the unknowns to be read. As this reaction is also seriously affected by water, it is important that all glassware used in this test be perfectly dry.

DISCUSSION

A number of experiments were conducted to check the accuracy of the method. The first step was to determine whether this method of extraction removes all the cholesterol from the blood. A series of extraction tubes were set up with 0.5 c.c. of blood in each. Cholesterol in increasing amounts were added to each successive tube. As is shown in Table I, the added cholesterol was completely recovered within experimental error.

TABLE I

RECOVERY OF ADDED CHOLESTEROL FROM BLOOD

TUBE	ORIGINAL CHOLESTEROL CONTENT IN 0.5 C.C. OF BLOOD (MG.)	ADDED CHOLESTEROL (MG.)	CHOLESTEROL IN EACH TUBE (MG.)	CALCULATED AMOUNT OF CHOLESTEROL IN 100 C.C. OF BLOOD (MG.)	AMOUNT OF CHOLESTEROL RECOVERED (MG.)
1	0.88	None	0.88	176	176
2	0.88	0.4	1.28	256	249
3	0.88	0.8	1.68	336	330
4	0.88	1.2	2.08	416	422
5	0.88	1.6	2.48	496	487
6	0.88	2.0	2.88	576	565

In another experiment cholesterol equivalent to 1,200 mg. per 100 c.c. of blood was dissolved in petroleum ether and precipitated with digitonin. The petroleum ether filtrate was evaporated and replaced by chloroform. Treatment with acetic anhydride and sulfuric acid failed to develop any trace of color, thus showing that the digitonin solution as used in this method completely precipitates free cholesterol.

RECOVERY OF CHOLESTEROL ESTERS

In this experiment cholesterol esters in increasing amounts were added to blood. The cholesterol esters were extracted from 100 c.c. of pooled blood mixed in a large flask with anhydrous sodium sulfate and extracted with chloroform.

TABLE II

RECOVERY OF ADDED CHOLESTEROL ESTERS FROM BLOOD

TUBE	ORIGINAL ESTER CONTENT IN 1.5 C.C. OF BLOOD (MG.)	ADDED ESTERS (MG.)	TOTAL ESTERS (MG.)	CALCULATED AMOUNT OF ESTERS PER 100 C.C. OF BLOOD (MG.)	CHOLESTEROL ESTERS RECOVERED PER 100 C.C. OF BLOOD (MG.)
1	0.93	None	0.93	62	61
2	0.93	0.5	1.43	95	94
3	0.93	1.0	1.93	129	131
4	0.93	3.0	3.93	262	256
5	0.93	5.0	5.93	395	388

The free cholesterol was completely removed with digitonin, and the cholesterol esters were dissolved in chloroform. Fifty-seven milligrams of cholesterol esters were obtained, as shown by a quantitative estimation. Varying amounts of these cholesterol esters added to samples of blood were completely recovered. This is shown in Table II.

More than four hundred determinations were performed by this method on blood coming to the laboratory for routine chemical examination. The average figure for total cholesterol in normal blood was about 180 mg., and the average ester content about 65 mg. per 100 c.c. of whole blood.

SUMMARY

A simple and accurate method for the determination of total cholesterol and cholesterol esters in blood is described. The blood is mixed with anhydrous sodium sulfate and extracted with chloroform. One aliquot portion of this extract is used for the determination of cholesterol esters after removing the free cholesterol with digitonin. The extracts are treated with acetic anhydride and sulfuric acid and compared colorimetrically with a standard cholesterol solution.

REFERENCES

1. Bloor, W. R., and Knudsen, A.: The Separate Determination of Cholesterol and Cholesterol Esters in Small Amounts of Blood, J. Biol. Chem. **27**: 107, 1916.
2. Sperry, W. M.: The Relationship Between Total and Free Cholesterol in Human Blood Serum, J. Biol. Chem. **114**: 125, 1936.
3. Kelsey, F. E.: Determination of Cholesterol, J. Biol. Chem. **127**: 15, 1939.
4. Bloor, W. R.: The Determination of Cholesterol in Blood, J. Biol. Chem. **24**: 227, 1916.
5. Myers, V. C., and Wardell, E. L.: The Colorimetric Estimation of Cholesterol in Blood, With a Note on the Estimation of Coprosterol in Feces, J. Biol. Chem. **36**: 147, 1918.
6. Leiboff, S. L.: A Simplified Method for Cholesterol Determination in Blood, J. Biol. Chem. **61**: 177, 1924.
7. Breh, F.: The Use of Air-Cooled Condensers in Blood Cholesterol Determinations, J. Lab. & Clin. Med. **24**: 635, 1939.

MICROCHEMICAL DETERMINATION OF YELLOW PHOSPHORUS*

LIEUT. SIDNEY KAYE,† U. S. ARMY

PHOSPHORUS is one of the more commonly known elements. It is, therefore, fitting and important that accurate, specific, and sensitive methods for its detection and determination should be available. The literature describes several satisfactory procedures based on gravimetric, volumetric, and colorimetric analyses. A new method for the confirmation and quantitative determination is herein described, using a spot test color reaction which is confined to a standard area the color intensity of which is proportional to the concentration of phosphorus present. This new method is not only specific and sensitive but also very simple. Its principle is based upon the formation of phosphine which in turn easily reduces a mercuric bromide test paper, yielding a canary yellow color.

Phosphorus may exist in several forms; the best known of these are yellow and red. Red phosphorus is considered by most authorities as nontoxic, whereas yellow phosphorus is very toxic and 0.1 Gm. has been recorded to be fatal. The method outlined here is applicable to yellow phosphorus only and is satisfactory, since our interest is primarily from a toxicologic viewpoint. Yellow phosphorus is used commonly in rat poison pastes and acute poisoning is usually accidental, but suicides and homicides have been recorded wherein phosphorus had been used.

Phosphorus may remain in the system for several days, but the greater the delay in obtaining the specimen, the less is the possibility of detecting it. The procedure of isolating it for analysis depends upon its volatility. Stomach contents are best suited for analysis.

A brief summary of the toxicology of phosphorus is included in this paper for completeness.

Symptoms.—Progressive pain and burning along the gastrointestinal tract, with belching, choking, vomiting, and diarrhea are present. The following additional symptoms are frequently noted: garlic odor on the breath, jaundice of the skin, bleeding from the nose and intestines, muscular paralysis and impairment of the special senses. Terminally, stupor, delirium, and coma occur. Death occurs usually within two to six days.

Treatment.—Wash the stomach with 1 per cent potassium permanganate and large amounts of suspended charcoal. If done quickly, this may be effective.

Post-Mortem Pathology.—There is corrosion of the mucous membrane of the gastrointestinal tract, with fatty degeneration and acute yellow atrophy of the

*From the Puerto Rican Department Laboratory, San Juan, Puerto Rico, Lieutenant Colonel V. H. Cornell, M.C., commanding.

†First Lieutenant, Sanitary Corps.

Received for publication, May 16, 1942.

liver. The stomach contents, as a rule, have the odor of garlic, and if viewed in the dark, give off a greenish phosphorescent light.

Chemical Analysis.—The following qualitative tests have been used to identify phosphorus positively:

1. Odor: The garliclike odor may be recognized on opening the stomach.

2. Scherer's test: A portion of the finely divided material is placed in an Erlenmeyer flask and suspended in distilled water. Two strips of filter paper are suspended over the suspected sample, one impregnated with silver nitrate and the other with lead acetate. The contents are heated at about 50° C. on a water bath for approximately an hour. If the silver nitrate paper turns black, either phosphorus or hydrogen sulfide may be present. If both papers turn black, hydrogen sulfide is present and phosphorus may also be present. The fact that the silver nitrate paper turns black is not conclusive proof of the presence of phosphorus, since any volatile reducing agent, such as formaldehyde, may also give the same reaction. Therefore, this test is more important in indicating the absence of phosphorus rather than its presence.

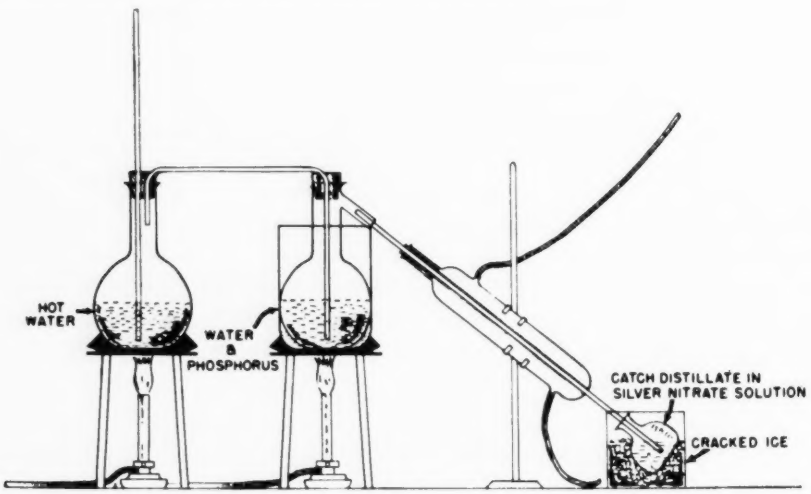


Fig. 1.

A NEW QUANTITATIVE TEST FOR PHOSPHORUS

A weighed aliquot of the sample is transferred to a steam distillation apparatus with about 100 ml. of water and an ice-cooled receiver is used to collect the distillate, as illustrated in Fig. 1. The light from the Bunsen burner is shielded by a sheet of asbestos to prevent any interference in the visual detection of the phosphorescence emitted. The distillation is performed in the dark, and the distillate is collected in a small volume of 0.5 N silver nitrate, which is cooled in a 125 ml. Erlenmeyer flask immersed in an ice bath. Greenish phosphorescence* may be seen as the phosphorus vapors reach the condenser. As little as 60 gamma† in 200 Gm. of material give a good phosphorescence. However, in the presence of alcohol, ether, benzene, turpentine, phenols, chloroform, essential oils, oxidizing agents, mercury, copper, and silver there may be a marked

*Mitscherlich's phosphorescence test.

†One gamma = 0.000001 Gm. or 0.001 mg.

diminution or complete absence of the phosphorescence. The distillation is continued until about 75 ml. are collected. As the first traces of phosphorus come over into the silver nitrate solution, a black precipitate of silver phosphide forms. After about 75 ml. have been collected, it will be noticed that on continued distillation no further precipitate forms. Silver phosphide is a heavy precipitate and settles rapidly. The supernatant liquid is siphoned off without disturbing the precipitate, leaving a small amount of liquid behind. The precipitate is then washed in the flask with several portions of distilled water to remove the last traces

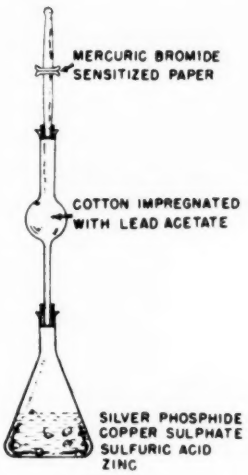


Fig. 2.

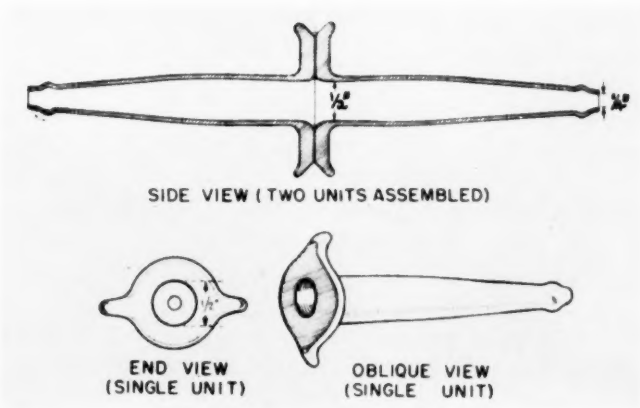


Fig. 3.

of any adhering silver nitrate. The supernatant fluid is again siphoned off. Two washings usually suffice. The washings are tested for silver ions by a chloride solution. If the precipitate is scant in the receiving vessel, make up volume to 50 ml. with water. If the precipitate is heavy, dilute to a larger known volume with water. Swirl the solution well so as to divide evenly the precipitate, withdraw a recorded small aliquot portion, and add it to a previously prepared modified Gutzeit apparatus (Fig. 2). This is essentially a 125 ml. Erlenmeyer flask containing some arsenic-free zinc, several drops of 5 per cent copper sulfate solution to

activate the process, and 25 to 40 ml. of 20 per cent sulfuric acid. The amount of sulfuric acid used should be sufficient to dissolve the precipitate in the aliquot added; excess does not interfere. The stopper in the flask supports a small calcium chloride drying tube into which is plugged some cotton impregnated with lead acetate. Above this tube is attached a set of flanges* between which is placed a disk of mercuric bromide† sensitized filter paper. On contact of the silver phosphide with the hydrogen generating solution, phosphine is liberated. The generating flask is kept warm on a water bath at about 50° C. Phosphine travels upward and is purified from hydrogen sulfide by passage through the lead acetate cotton. On reaching the flanges when it contacts the moistened mercuric bromide paper, a yellow color results which is directly proportional to the phosphine produced. Since the bores of the several flanges are uniform, comparisons can be made with phosphorus standards prepared in a similar manner. This color reaction is sensitive to less than 10 gamma of phosphorus. To be certain that the zinc and other reagents are arsenic and phosphorus free before adding the silver phosphide, hydrogen is liberated for some time and then the filter disk is examined. If this proves negative, the silver phosphide is then added and the flanges are put in place. The generator is allowed to run for about half an hour. The yellow color standards, when once prepared, are fairly stable and can be kept for several months if dry.

Other Uses for the Microchemical Flange.—The flanges described promise to be of great value for quantitative determinations in both gas and solution analyses where the intensity of a color reaction, which is proportionate to known amounts of the substance concerned, can be recorded.

Their use is being studied for the determination of sulfides, cyanides, alcohol, phenol, and other volatiles. For substances in solution which give brilliant graded colors with a detector, the flanges also have proved successful. A rapid method for the detection of lead in urine has been perfected and will be published later. The flange device was used in the Chemical Laboratory at Bellevue Hospital, New York City, while seeking more accurate and sensitive methods for the determination of arsenic. A paper on the subject is in progress and will be published from Bellevue Hospital.

Its broader application has been successfully demonstrated in the past few months at the Puerto Rican Department Laboratory.

SUMMARY

A brief review of the toxicology of phosphorus poisoning is given.

A new specific method for the quantitative microdetermination of phosphorus is described.

The principle involved depends upon trapping the phosphorus as silver phosphide, generating the phosphine by hydrogenation, and measuring the phosphine generated on a mercuric bromide filter disk. The color developed is a

*The flanges may be purchased cheaply from Eck & Krebbs or E. Machlett & Sons, both of New York City.

†Mercuric bromide sensitized paper is prepared by cutting round disks of Whatman No. 40 filter paper which are impregnated with 5 per cent mercuric bromide in 95 per cent alcohol for about five minutes. After shaking the droplets off, a disk is fastened between the flanges with a rubber band.

measure of the amount of phosphorus present and is determined by comparison with standards similarly prepared.

The microchemical flanges are a new device which may be used in a similar manner for other determinations, some of which have been mentioned.

REFERENCES

1. Wolf, L., Dusing, W., and Martes, A.: Phosphorus, Mikrochem. **18**: 185, 1935.
2. Bamford, Frank: Poisons, New York, 1941, The Blakiston Co.
3. Goldbaum, Leo and Umberger, Joseph: New York, Personal communications.
4. Thorne, P. C. L., and Ward, A. M.: Fritz Ephrain Inorganic Chemistry, New York, 1939, Nordemann Publishing Co., Inc.

QUALITATIVE STUDIES OF THE BILIRUBIN IN BODY FLUIDS*

II. A Comparison of the Direct Diazo Reaction by the Photoelectric Colorimeter, the Three Test Tube Method, and the Oxidation Test in Xanthochromic Spinal Fluid

Georg Lepehne, M.D., Boston, Mass.

IN A PRECEDING paper[1] qualitative studies of the bilirubin in blood serum by comparison of the direct diazo reaction using the photoelectric colorimeter, the three test tube method, and a new qualitative oxidation test for differentiation of the various types of jaundice were reported. In this paper similar studies concerning the bilirubin of xanthochromic spinal fluid will be presented.

METHOD

The xanthochromic spinal fluid was examined by the methods described in the preceding paper: the direct diazo three test tube method with use of caffeine-sodium salicylate,[2] the direct diazo photoelectric colorimeter method of Malloy and Evelyn,[3] and the qualitative oxidation test tube method[1] using a reagent consisting of nitric acid and sodium nitrite.

RESULTS

In 8 specimens of xanthochromic spinal fluid (hemorrhages, tumor, operation of the brain) the direct diazo photoelectric examination and the three test tube examination were done. In addition 7 specimens were examined by the three test tube method alone. In all 8 specimens the photoelectric direct diazo curves showed a definitely delayed, very slow ascending type of curve. On two specimens with the total bilirubin content of 2.28 mg. per 100 c.c. and 3.79 mg. per 100 c.c., respectively, in which the three test tube method revealed a biphasic-delayed direct diazo reaction, curves of delayed type were obtained too (see Fig. 1). These curves are very similar to those found in sera with delayed direct diazo reaction (hemolytic jaundice, jaundice of the newborn, etc.).[1] They are in clear contrast to the prompt direct curves obtained in serum of obstructive or

*From the Department of Medicine, Boston University School of Medicine.
Received for publication, August 13, 1942.

catarrhal jaundice. The indirect diazo reaction following the addition of methyl alcohol gave the usual steeply ascending curves (see Fig. 1). In order to express the significance of the direct curves in ratios the one-minute and the thirty-minute readings of the direct curves were compared with each other and with the one-minute and with the thirty-minute values of the indirect curves, as in the first paper (see Table I). On the average, the direct curve reached in one minute was 15.4 per cent, in thirty minutes, 36.2 per cent only of the total bilirubin content. The corresponding ratios in sera with delayed direct diazo reaction reported in the preceding paper were very similar, while the ratios in the prompt reacting sera of obstructive and catarrhal jaundice were entirely different.

TABLE I

COMPARISON OF QUALITATIVE AND QUANTITATIVE REACTIONS
XANTHOCHROMIC SPINAL FLUID

NO.	TOTAL PROTEIN (MG. PER 100 C.C.)	TOTAL BILIRUBIN (MG. PER 100 C.C.)	1 MIN. DIRECT/ 30 MIN. INDIRECT (%)	1 MIN. DIRECT/ 1 MIN. INDIRECT (%)	1 MIN. DIRECT/ 30 MIN. DIRECT (%)	30 MIN. 30 MIN. DIRECT/ INDIRECT (%)
1	168	0.4	20.5	22.7	55.4	37.0
2	219	0.6	17.7	20.5	43.9	40.9
3	570	0.8	12.5	18.2	39.5	31.3
4	116	0.4	12.2	14.6	37.4	32.7
5	192	0.6	27.1	35.5	54.0	50.1
6	264	1.0	18.7	29.5	26.0	48.0
7	1,500	2.3	10.1	15.3	30.7	32.9
8	710	3.8	4.3	6.1	26.9	16.7
Average			15.4	20.3	39.2	36.2

The three test tube direct diazo reaction was carried out in 15 specimens of xanthochromic spinal fluid, and in 13 specimens the reaction was definitely delayed. The first slight redness developed in 10 cases within an average time of five and one-half minutes. In 5 cases the red color had not developed within one hour. The change to purple color, described in the first communication, was not seen. The longest period of observation was two and one-half hours. The two specimens with the high bilirubin level (Table I, Nos. 7 and 8) showed by this method, in contrast to the delayed photoelectric curves, a biphasic-delayed direct diazo reaction. Immediately after addition of the diazo reagent a trace of redness developed, but the red color increased very slowly. The comparison of the three test tube method with the photoelectric method shows that the photoelectric readings are higher than one should expect according to the very slow development of the red color in the test tube method. This fact seems to depend on the greater accuracy of the photoelectric method to detect the color.

Vaughan and Hubbard[4] claim that every xanthochromic spinal fluid gives a "prompt" direct diazo reaction when the bilirubin content exceeds 0.3 mg. per 100 c.c. But one must consider that their expression "prompt" direct diazo reaction includes also biphasic-prompt and biphasic-delayed direct reactions[1] according to the usual nomenclature, so that their results are confusing. In our specimens a really prompt direct diazo reaction was not observed by our methods in spite of the fact that the bilirubin content was higher than 0.3 mg. per 100 c.c. (Table I). These authors also believe that the type of the direct diazo reaction in xanthochromic liquor with a bilirubin content below 0.3 mg.

per 100 c.c. depends upon the total protein content. In our cases with higher bilirubin values the total protein content ranged from 49 mg. to 3,720 mg. per 100 c.c. and did not influence the type of the reaction.

The oxidation test was made in 14 specimens of xanthochromic spinal fluid. In 12 cases the test was "negative" (a straw color developed or the color did not change at all). In the two specimens with higher bilirubin content, which gave a biphasic-delayed direct diazo reaction, the oxidation test was "diminished-delayed" (a slight yellowish-green color appeared twenty to thirty seconds after addition of the reagent). In order to control the oxidation test a drop of serum of obstructive jaundice was added to a specimen of colorless liquor. A real green color developed immediately ("prompt" oxidation test).

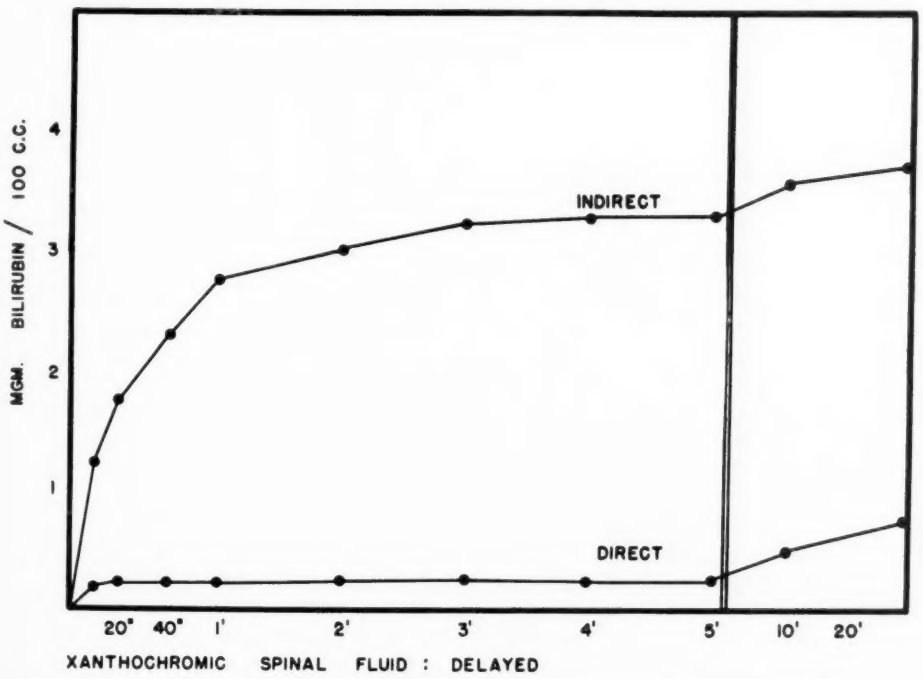


Fig. 1.—"Delayed direct diazo reaction" in a case of xanthochromic spinal fluid.

DISCUSSION

These examinations reveal that the bilirubin in xanthochromic spinal fluid gives the same qualitative reactions as the bilirubin in sera of hemolytic jaundice, of jaundice of the newborn, and of the nonhemolytic familial jaundice. These results support the theory that bilirubin giving such reactions is of anhepatic origin. Bilirubin in the blood of this type apparently did not pass the excreting liver cells. The liver cells change the direct diazo reaction from delayed to prompt (van den Bergh,[5] Aschoff,[6] McNee,[7] Lepehne,[8] Eppinger,[9] and other authors). The described examinations also reveal, as did the examinations on jaundiced serum,[1] that even in xanthochromic spinal fluid a really "negative" direct diazo reaction does not exist, when observation time is long enough. The direct diazo reaction can be, generally speaking, either prompt, biphasic, or delayed, but not negative.

SUMMARY

The bilirubin of xanthochromic spinal fluid was examined by the direct diazo three test tube method, by the direct diazo photoelectric method, and by the oxidation test. The direct diazo reaction was delayed or biphasic-delayed, and the oxidation test negative or diminished-delayed. The total protein content did not influence the type of the reaction. This bilirubin is of the same type as that in the blood serum in hemolytic jaundice and jaundice of the newborn.

REFERENCES

1. Lepehne, Georg: Qualitative Studies of the Bilirubin in Body Fluids. I. A Comparison of the Direct Diazo Reaction by the Photoelectric Colorimeter, the Three Test Tube Method and the Oxidation Test in Serum, J. Lab. & Clin. Med. 27: 1447, 1942.
2. Lepehne, Georg: Die klinische Bedeutung der Bilirubinbestimmung im menschlichen Blutserum, Deutsche med. Wchnschr. 49: 641, 1923.
3. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin With the Photoelectric Colorimeter, J. Biol. Chem. 119: 480, 1937.
4. Vaughan, S. L., and Hubbard, R. S.: Van den Bergh Reaction of Bilirubin in Xanthochromic Spinal Fluid, Proc. Soc. Exper. Biol. & Med. 32: 618, 1935.
5. van den Bergh, Hijmans A. A.: Der Gallenfarbstoff im Blute, Leiden und Leipzig, 1918.
6. Aschoff, Ludwig: Ueber Bildungs- und Ausscheidungsstörungen der Gallenfähigen Substanzen (Dyscholie), besonders des Gallenfarbstoffs (Ikterus), Acta path. et microbiol. Scandinav. 5: 338, 1928.
7. McNee, J. W.: Gibt es einen hämolytischen Icterus? Med. Klin. No. 9, 1125, 1913. Experiments on Hemolytic Icterus, J. Path. & Bact. 18: 325, 1914.
8. Lepehne, Georg: Pathogenese des Ikterus, Ergebn. d. inn. Med. u. Kinderh. 20: 221, 1921.
9. Eppinger, Hans: Hepato-lienale Erkrankungen, Berlin, 1920.

A SIMPLE METHOD FOR DETERMINING PLASMA DENSITY AND PLASMA PROTEIN CONCENTRATION*

Eric Ponder, M.D., D.Sc., Mineola, L. I.

IN THE course of an investigation on water balance and plasma protein concentration I have had occasion to make large numbers of plasma protein determinations by a modification of the Linderstrøm-Lang method for measuring density, which is so convenient and accurate that it is worth while drawing attention to it.

The only apparatus required is a glass cylinder of 100 to 200 ml. capacity, graduated in milliliters. At least every 10 ml. graduation mark should run all around the cylinder, so as to enable one to avoid parallax in making readings. The cylinder is placed at eye level on a well-lit shelf shielded from drafts, in a room in which rapid changes of temperature do not occur. It is half-filled with a brombenzene-kerosene mixture of density 1.0485, made by mixing 35 ml. of brombenzene and 65 ml. of kerosene (density of brombenzene = 1.497; density of kerosene = 0.807). On top of this is layered about 50 ml. of a lighter mixture of density 1.0210, made up of 31 ml. of brombenzene and 69 ml. of kerosene. A rod, with a ring which just fits inside the cylinder at right angles to it, is moved gently up and down about a dozen times in the region of the

*From the Nassau Hospital, Mineola.
Received for publication, August 25, 1942.

interface between the heavier and the lighter fluids, and in this way a remarkably even gradient of density is produced, usually between the 20 ml. and the the 80 ml. marks on the graduation of the cylinder.

A series of standards are prepared from the commercially supplied potassium sulfate standard of density 1.0551[1] by the addition of distilled water in proportions indicated in Table I. These standards are kept in dropping bottles with well ground-in pipettes. A drop of each standard is dropped into the fluid in the cylinder, through which it descends until it reaches a level with a density the same as its own. The drop is formed on the end of the pipette, dipping below the surface of the fluid; on withdrawing the pipette, the drop breaks off. Its size is not very important, but 5 to 15 c.mm. is a convenient size. In this way the variations in the density gradient in the cylinder are marked off by the position of the six drops of known density, which correspond to six readings on the scale on the cylinder. In making the readings, care must be taken to avoid parallax. If the densities of the drops are plotted against their position on the scale, a remarkably good straight line usually results, showing that the density gradient is linear. Even if it is not, the graph can be used for finding the density of the gradient at any level. If the gradient changes, as it may on account of variations in temperature, etc., the positions of the standard drops change with it, and they always mark the six positions of their own densities.

TABLE I

D = 1.0551 ML.	WATER ML.	D OF MIXTURE	CORRESPONDS TO PROTEIN, GM. PER 100 C.C.
18.0	12.0	1.0330	8.89
16.5	13.5	1.0303	7.96
15.0	15.0	1.0276	7.04
13.5	16.5	1.0249	6.11
12.0	18.0	1.0221	5.17
10.5	19.5	1.0193	4.23

If a drop of plasma from heparinized blood is allowed to fall through the fluid in the cylinder in the same way, it will stop at a position in the density gradient, which corresponds to its own density. A reading of this position on the scale and reference to the graph for the positions of the standard drops will give its density immediately, and its protein content can be found from the density in the usual way from tables, or from a graph prepared by plotting the last two columns of Table I. Since the lipoids of the drop and the brombenzene and kerosene are not mutually altogether insoluble, the drop tends to sink slowly from the position which it first assumes, and so its position should be read after a standard time, e.g., one minute from the moment it is dropped into the cylinder. For the same reason, small drops are better than large ones, and 5 to 10 c.mm. is a convenient size. I use medicine droppers drawn out into capillary pipettes for forming the drops.

When some experience has been gained, the method will be found to give values for plasma protein concentration correct to ±0.1 Gm. per 100 c.c. This is not as high a precision as is claimed for the falling-drop method, but both methods have the weakness that what they measure is plasma density, which depends on protein concentration only when the lipoid content is constant, and

so a precision sufficient to measure ±0.0003 in the figure for density is enough for all clinical purposes. The method is convenient in practice, and at least 20 determinations can be made before the fluids need replacement and a new gradient has to be formed. Unwanted drops can be removed by touching them with a small piece of wet filter paper attached to the end of a capillary pipette. The drops adhere to the paper and can be pulled up out of the brombenzene-kerosene mixture.

REFERENCE

Linderstrøm-Lang, K.: Distribution of Enzymes in Tissues and Cells, Harvey Lecture 34: 214, 1939.

Solutions of standard density used in the falling-drop method for determining density are supplied by Eimer and Amend, New York, and other scientific apparatus houses.

MEDICAL ILLUSTRATION

A SIMPLE TECHNIQUE FOR TIME LAPSE CINEMICROGRAPHY*

ADRIANUS PIJPER, M.D., D.SC., AND LAURIE B. POOLE
PRETORIA, SOUTH AFRICA

PURPOSE OF THE METHOD

NEW methods lead to new findings. Cinematography in many laboratories is still a novelty. Photographing the subject through a microscope at ordinary speed may show nothing beyond what the eye has noticed already, although even here the film often records something that has escaped detection by the eye alone. Slow motion cinemicrography analyzes movements too rapid to be observed by the human eye. In time lapse cinematography and cinemicrography we have methods that make visible what no human eye has the patience or the facility to observe.

Time lapse cinematography is the process of taking pictures at a slower rate than the one at which they are eventually projected. Movements too tedious or too slow to be watched can thus be condensed into processes which are easily followed on the screen. Audiences are familiar with the method as applied, for instance, to the unfolding of a flower. In time lapse cinemicrography the same method is used but the pictures are taken through a microscope.

New methods can only bring results in proportion to the frequency with which they are used. Time lapse cinemicrography so far has not been applied widely. The hesitation to put into regular use a method so full of possibilities is largely caused by technical considerations. We believe that the technical difficulties of the method are overestimated. Considerable work in this field has been done by Comandon. Splendid apparatus has been built by Rosenberger,[5] Harris,[2] and by Roger,[6] but we feel that excellent results can be obtained by much simpler methods. In this paper we wish to show that time lapse cinemicrography is within the reach of most biologic laboratories. Our apparatus has been built by the simplest possible means and can be set up on an ordinary laboratory bench.

SPEED CONTROL

The chief part of the speed control is an ebonite disk, measuring 6½ inches in diameter (Figs. 1*A* and 2*A*), which revolves once a minute. It is driven by an ordinary electric clock motor (Fig. 2*B*), which is operated on 60 cycle alternating electric current. There are 60 small equidistant holes drilled through this disk on a circle near the periphery. Tapering steel pins can be pushed into these holes. As the disk rotates, these pins make electric contacts which activate the shutter between the lamp and microscope, and also drive the camera and a counter.

*From Dr. A. Pijper's private laboratory.
Received for publication, July 20, 1942.

It is obvious that the longest lapse of time available with this apparatus is one minute, and the shortest time is a second. In addition, one can work at any number of seconds in between these two values by changing the number of pins on the disk. Up to the present we have found this time control ample for our requirements.

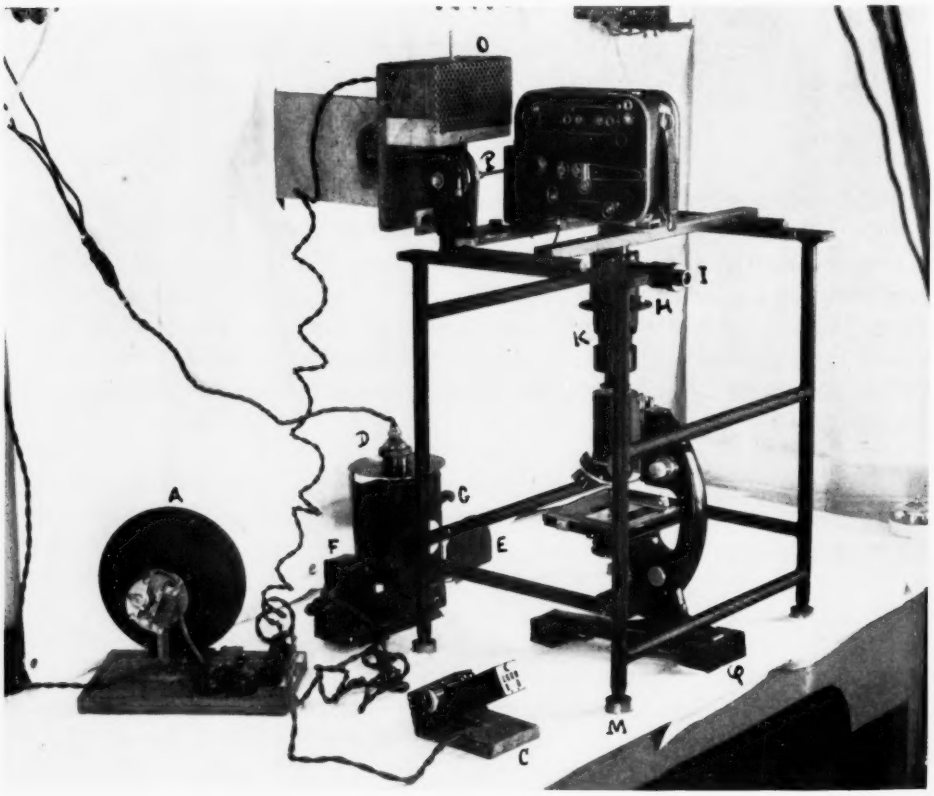


Fig. 1.—Side view of the apparatus.

A diagram of the wiring is given in Fig. 3. The steel pins make contact by depressing the metal tongues as they go through the lowest point of their rotation. The tongues are placed on top of one another and are arranged so that the one that activates the shutter between the lamp and microscope keeps this shutter open long enough for the tongue that works the camera to expose the film. The counter (Figs. 1*C* and 2*C*) is added in order to check the number of exposures made but is not an essential part of the apparatus.

SOURCE OF LIGHT

An Ediswan Pointolite lamp of 100 C. P. is used (Figs. 1*D* and 2*D*). A stronger lamp would be better but is not procurable under present conditions. An arc lamp, of course, is too unsteady for this kind of work. The shutter between the lamp and microscope (Figs. 1*E* and 2*E*) is made of light metal and is essential because it prevents exposure of the microbes to continuous light. It is lifted by a solenoid (Figs. 1*F* and 2*F*) and drops by its own weight. The shut-

ter can be kept raised permanently by pushing a lever (Fig. 1*G*) to the left. This is useful for making adjustments to the microscope.

THE MICROSCOPE

We use the large Zeiss stand FCG, as shown in Figs. 1 and 2, but any other heavy stand will serve. Since the dark field is the method of choice for this class of work, we use the Siedentopf cardioid condenser. To date we have worked mainly with fungal material, and a suitable combination of lenses was found in the Zeiss apochromat objective ×60 with iris and the Zeiss compensating eyepiece ×7. We have also used the Zeiss compensating eyepiece ×10. The distance between eyepiece and film is about 15 cm. These combinations resulted in magnification on the film of ×200 and ×310, respectively.

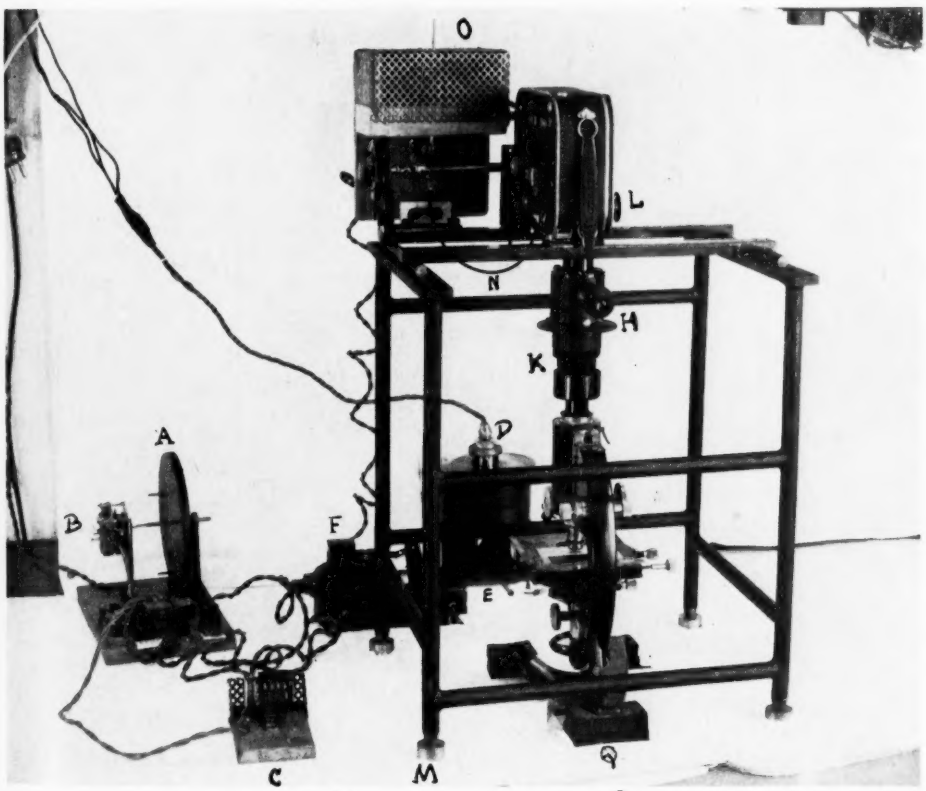


Fig. 2.—Front view of the apparatus.

THE PREPARATION ON THE SLIDE

For studying the growth of monilias, yeasts, and other fungi we place a small drop of a fairly thick suspension of spores on a microscope slide. A drop of melted Sabouraud agar cooled down to about 50° C. is added, and a cover slip is dropped on rapidly. Previous setting of the agar and moving of the cover slip must be avoided, since these lead to the formation of channels in the agar filled with thin fluid in which the spores swim about instead of lying still in solid agar. Petroleum jelly is applied around the cover slip. The microscope is then adjusted and the proper field is found in the usual way.

THE CAMERA

Our camera is a standard Movikon, with the lens removed and replaced by the beam splitter made by Zeiss for such purposes (Figs. 1*H* and 2*H*). Of course, any other make, or even a homemade one, will do. The one we use gives an additional magnification which is helpful in focusing. A suitable cap should be placed over the eye lens of the beam splitter (Fig. 1*I*), otherwise, as Barnard has pointed out,[1] extraneous light may reach the film. A lightproof connection between the microscope and camera is provided by a black velvet skirt (Figs. 1*K* and 2*K*) which fits into the sleeve around the eyepiece. The microscope and camera should not touch one another.

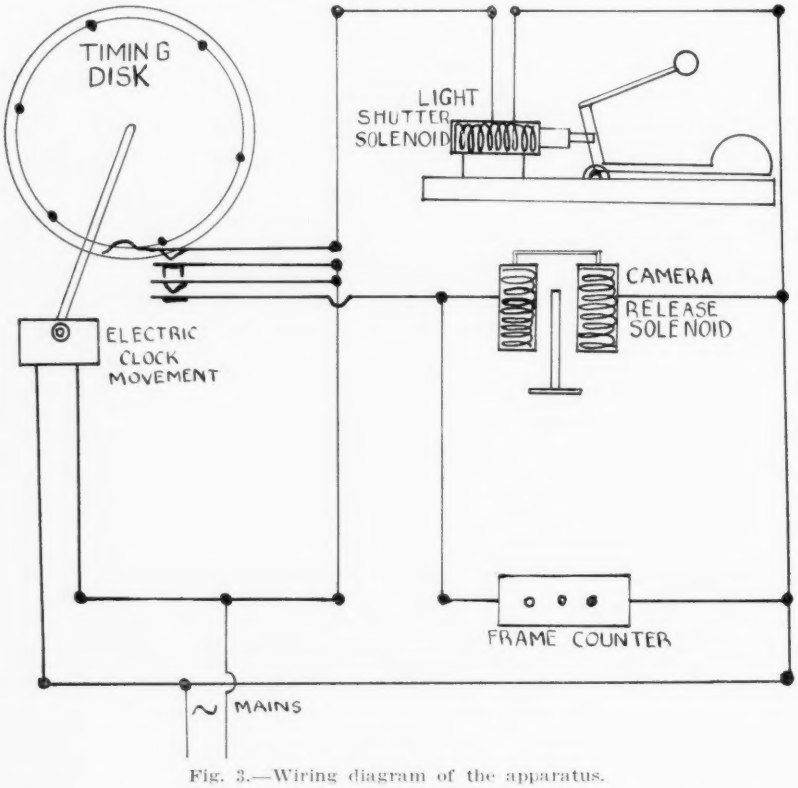


Fig. 3.—Wiring diagram of the apparatus.

The camera is supported on two horizontal flat iron bars, as seen in Figs. 1 and 2. It rests only on the front one and the posterior bar carries an upright piece of metal to which the camera is clamped by means of a clamping screw. This screw threads into the hole at the base of the camera provided for that purpose. The two horizontal flat bars also merely rest on the top of the iron stand which surrounds the microscope, as is shown in Figs. 1 and 2. From these illustrations it is seen that the camera can slide forward and backward with the two flat bars along the two round iron bars included purposely. This is necessary for accurate centering over the microscope. Slight movements to the side during centering are best executed by changing the position of the microscope. Centering is done through the beam splitter, but the beam splitter itself should be centered by looking at the picture on the film through the focusing magnifier which belongs to the camera (Fig. 2*L*).

The stand around the microscope consists of four uprights, which are connected by sufficient crossbars to keep the structure rigid and to allow access to the microscope. The microscope can be handled easily with the stand in place. It can, however, be removed and be replaced again by means of the four brass cups (Figs. 1*M* and 2*M*) into which the conical ends of the four uprights fit tightly. These cups are screwed to the bench.

The Movikon camera possesses the essential single picture mechanism and is conveniently set in motion by means of a wire release (Fig. 2*N*). This wire release is activated through a solenoid (Figs. 1*O* and 2*O*), which is attached to a wall bracket and does not touch the stand or the camera.

Fig. 4.

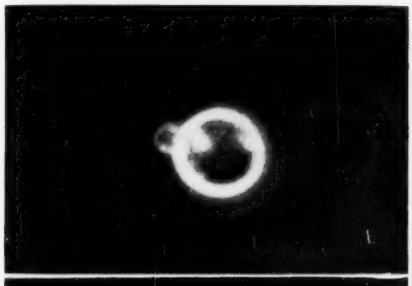


Fig. 5.

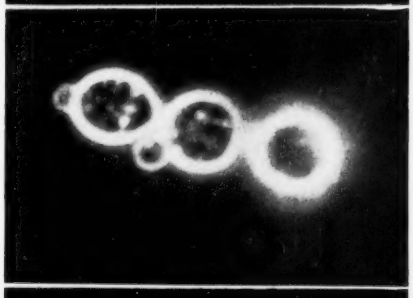
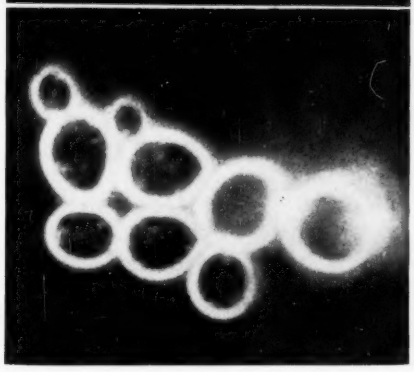


Fig. 6.



Figs. 4, 5, and 6.—Frames from a film of budding Monilia cells.

The single picture mechanism of this camera does not allow an exposure longer than $\frac{1}{25}$ second. This is ample for ordinary work but is insufficient for cinemicrography with a light source of 100 C.P., unless the lowest magnifications are used. In looking for a means to lengthen the exposure, we found that the Movikon camera has a shaft or rewinding spindle for making mixes. This shaft protrudes through the housing and makes a half revolution during each exposure. It was decided to slow down this shaft by attaching to it the dial

of an automatic telephone. The exposure was thus increased. Such a dial moves at a fixed speed which is controlled by a built-in governor. In Figs. 1 and 2 the dial is shown attached to an upright that is fixed to the posterior horizontal bar, to which the camera is attached in a similar manner. The dial center spindle is connected to the extended shaft of the camera by a universal coupling, a length of shafting, and a screw which is fitted onto the splined end of the coupling shaft. The splines and screw allow the dial to be disconnected quickly for winding the camera, but this cannot be done with the connection in place. By such an arrangement we found that the shutter action of the single picture mechanism was slowed down to $\frac{1}{5}$ second.

The use of the camera spring for moving the film eliminates the geared electric motors which are usually deemed essential for time lapse cinematography. Our plan prevents their vibrations. The disadvantage of this method is that the spring must be wound at regular intervals. One winding suffices for 20 feet of film, and at a speed of one picture a minute each winding lasts ten hours. Frequent inspections of the field are necessary to make sure that nothing untoward is happening. Therefore, the occasional winding of the spring is of no great consequence.

VIBRATION

The danger of spoiling cinemicrographic pictures by vibration is often overrated. For speeds of 16 frames a second, for instance, or even 64 frames a second, we have shown previously that it is quite safe to place both the camera and microscope on the same iron optical bench,[3, 4] provided the eyepiece of the microscope does not touch the camera and the spring of the camera is used to move the film. For our time lapse cinemicrography with its longer exposures we found it safe to place the whole outfit on an ordinary wooden laboratory bench, if the precautions mentioned were observed. The weight of the camera with its attachments holds it steady. In addition, the camera with the posterior horizontal bar and the telephone dial can be lifted up and put aside as one piece by simply undoing the wire release from the camera. The anterior horizontal bar is loose and thus the microscope is ready for ordinary use at a moment's notice.

There is still another feature to explain. We prevent vibration of the microscope by placing its base on soft rubber pads (Figs. 1*Q* and 2*Q*). It is held in place by a long fastening screw which goes through a hole in the wooden bench and reaches the base of the microscope from underneath. The hole through the bench is lined with soft sponge rubber, and there is a rubber washer separating the head of the fastening screw from the bench beneath. This arrangement allows slight movements of the microscope from side to side and assists in centering.

FILMING OF BUDDING MONILIA CELLS

By means of the apparatus described we have made a film of budding Monilia cells, from which frames are reproduced in Figs. 4, 5, and 6. In the film the budding cell of Fig. 4 can be seen to produce by repeated budding and separating, first, the three cells of Fig. 5, and finally the seven cells in Fig. 6. It shows that before budding takes place there is great activity of the particles that can be seen in the cells. Usually this is near the part of the wall that is

going to break down. The yielding of the cell wall is a surprisingly sudden process compared with the following development of the bud. It is again accompanied by great activity of the particles, which can be seen making their way into the new cell. As a rule, it is possible to keep in focus the communicating opening between the two cells. The cell wall of a new cell is at first much thinner than later. As soon as it reaches the thickness of the old cell, the opening closes and often the cells separate with a sudden jerk.

SUMMARY

A technique is described which enables biologic laboratories possessing ordinary equipment and the services of a skilled technician to make time lapse cinemicrographic 16 mm. films. The apparatus is put together from simple materials, can be used on an ordinary wooden laboratory bench, and can be assembled and taken apart in a few moments, so that the microscope remains available for ordinary use.

REFERENCES

1. Barnard, J. E., and Welch, F. V.: Practical Photomicrography, London, 1925.
2. Harris, P.: Automatic Operation of Ciné-Camera and Lighting Arrangements for Time Lapse Studies, Nature **142**: 834, 1938.
3. Pijper, A.: Microcinematography of the Motile Organs of Typhoid Bacilli, J. Biol. Photographic A. **8**: 158, 1940.
4. Pijper, A.: Microcinematography of the Agglutination of Typhoid Bacilli, J. Bact. **42**: 395, 1941.
5. Rosenberger, H.: Scientific Apparatus and Laboratory Methods: A Standard Microcinematographic Apparatus, Science **69**: 672, 1929.
6. Roger, H.: A New Camera Timer for Time Lapse Cinematography, J. Soc. Mot. Pict. Eng. **32**: 549, 1939.

THE MINIATURE CAMERA APPROACH TO VISUAL EDUCATION*

GEORGE FISKE JOHNSON, PH.D., STATE COLLEGE, PA.

IT IS NOW almost six years since the miniature camera first attracted the serious attention of educators in the United States as a new approach to effective visual instruction. The growing enthusiasm among the professions for this type of camera and its products as an aid in teaching and in recording scientific data is revolutionizing the photographic and visual aid programs of many institutions.

The possibilities of the miniature camera and related equipment in photographing medical subjects, especially in color, have been reviewed previously in this JOURNAL. Jeter and Hull[1] pointed out several years ago the value of using 35 mm. color film in miniature cameras as a means for securing 2 by 2 inch slides for illustrative purposes. "The pathologist and the pathologic department of every hospital should assume teaching responsibilities," they wrote. "We believe color photographs, as carried out by the simple, inexpensive method described, is now practical and, furthermore, adds greatly to the facilities of teaching."

*From the Agricultural Extension Service of the Pennsylvania State College.
Received for publication, July 21, 1942.

Tomlinson[2] recently called the attention of the medical profession again to miniature cameras, 35 mm. color film, 2 by 2 inch slides, illuminators for displaying color transparencies, and especially their application to photomicrography.

DeLucchi,[3] reporting on a survey of 55 medical school hospitals, clinics, biologic photographic departments, and medical laboratories, writes: "There is a very noticeable increase in the use of the smaller 2 by 2 inch Kodachrome slides (photographed usually in miniature cameras). . . . A numerical checkup will show that the demand has been so great that the smaller-sized slide is definitely and rapidly becoming part of the routinely produced material in a majority of biological laboratories. The field of photography as used to illustrate surgical procedure has also been invaded by the 2 by 2 inch Kodachrome slide. More and more this small size slide is being used to augment 16 mm. Kodachrome motion pictures, and in one department has replaced the motion picture to a large extent. It is felt that a series of pictures taken during the process of an operation are excellent for teaching. The cost of such a series is less than a motion picture covering the same procedure. It is to be noted that three out of four new photographic laboratories (less than one-year old) are using 2 by 2 inch slides. . . ."

Other writers, especially in the field of biology, such as Brubaker and Holinger,[4] Waller and Breedis,[5] Footer,[6] and Koch,[7] have recognized this new trend in photography and visual education and have recorded many valuable ideas and experiences.

I have been associated with the development of a visual aid service in the division of agricultural and home economics extension at the Pennsylvania State College for the past seven years. This service employs 200 county farm and home economic representatives and state-wide subject matter specialists who conduct demonstrations on better farm and home economic practices and assist with 10,000 educational programs in rural communities annually.

Our enthusiasm for the miniature camera was generated in 1937 when it was demonstrated that a $15 camera (more expensive now) taking the new 35 mm. color film could produce reasonably true-to-color transparencies at a film cost of 15 cents each. We saw in this simple, inexpensive equipment the means of improving the photographic and visual aid program for our workers. The reasons for this were: (1) the equipment was light and easily carried; this was an extremely important advantage because our workers were traveling educators; (2) the equipment was less expensive than that available in the past; (3) the miniature camera loaded with color film was more foolproof and more exhilarating in results attained than larger cameras with black-and-white film; (4) the unit cost of the visual aids secured was substantially less than that prevailing with either the standard lantern slide or 16 mm. motion pictures. We now have in our organization 107 miniature (35 mm. film) cameras costing from $15 to $175 each.

Slides, 2 by 2 Inches, Lead as Visual Aids.—How revolutionary recent improvements in visual aids have been in our case can be cited specifically. The number of standard-sized black-and-white lantern slides in use decreased from 5,000 in 1935 to fewer than 500 (used infrequently) in 1942, and in their place have come almost 25,000 of the new 2 by 2 inch slides taken with miniature

cameras, mostly in natural color. We produced more than five times as many color slides in four years (1937 to 1941) as standard-sized black-and-white slides in twenty years (1917 to 1936). Even more significant was the fact that a complete equipment change requiring the adapting of old projectors or the purchase of new projectors was necessary to effect this change-over in the type of slide used.

Fig. 1.—County Agricultural Agent Russell M. Smith, Forest County, Pa., with a portion of the 800 2 by 2 inch slides which he has produced and utilized more than 300 times in the past four years on a total equipment and film expenditure of less than $225.00.

Screens for showing pictures changed in character completely during this seven-year period. Most screens in 1935 were white cloth on which color slides would not show up clearly in the daytime in rooms that could not be darkened completely. Only one solution could be found and that was to buy the beaded surface type of screen. A few demonstrations were extremely convincing, so that today we have 82 beaded surface screens, 77 in county offices and 5 in the State College office. Each county has at least one of these screens, and 11 counties have two each. The folding base tripod screen is most popular.

The trend in the use of visual aids since 1935 is shown in Fig. 3. The use of 2 by 2 inch slides has increased from zero in 1936 to almost 50 per cent of all meetings in 1942; 16 mm. movies (mostly silent), of which we made over 150 reels, gained from 5 to 15 per cent; large lantern slides and film strips decreased from 25 to 1 per cent; visual aids other than projected pictures dropped slightly from 30 to 25 per cent; and meetings with no visual aids decreased from 35 to 10 per cent. It will be observed that the 2 by 2 inch slides are used in a larger percentage of meetings today than all forms of projected pictures employed only seven years ago.

The mistake is made of regarding the 2 by 2 inch slides as nothing more than the large lantern slide in a new dress. This transparency is a *new visual medium*. Because of its low film and processing costs each step in a process

can be recorded economically. Furthermore, the mechanics of the camera are so simple that a sequence of pictures can be taken quickly. In this sense the miniature camera is a cross between the motion picture camera and the more cumbersome cut film or film pack reflex or view camera. Likewise, when skillfully used in the lecture room the resulting pictures retain all advantages of the slide medium while acquiring some of the advantages of the motion picture.

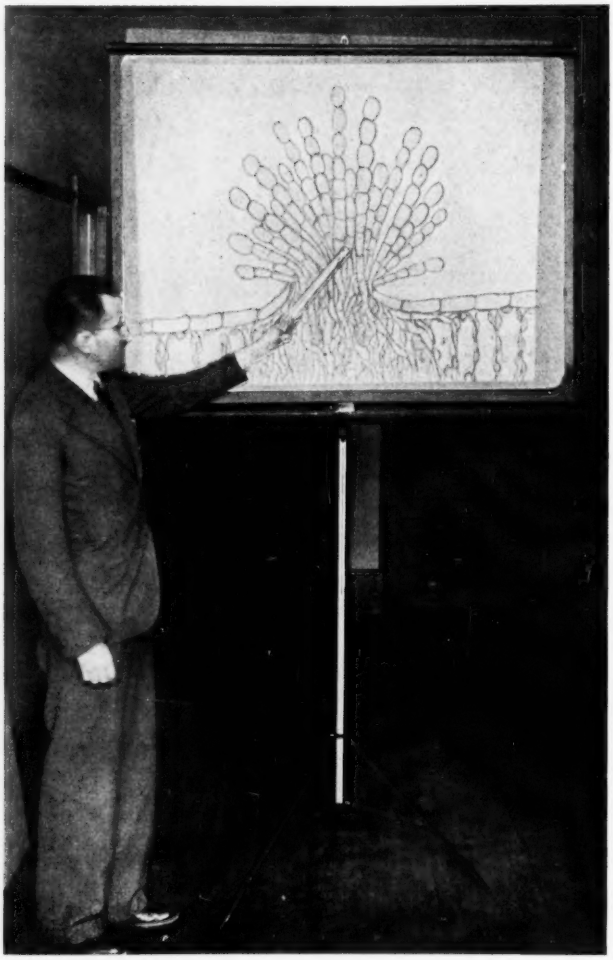


Fig. 2.—The folding base tripod beaded surface screen in use. Note that it can be elevated to shoulder height.

Advantages of Slides over Motion Pictures.—Although physical completeness is attained with a well-photographed motion picture of an operation or method demonstration, this does not mean that completeness of understanding is always reached, even when fully explained. The skillful lecturer can often take a well-photographed series of slides of this same operation or method demonstration and secure more complete and lasting understanding of the operation because: (1) the most critical stages or steps in the operation can be retained clearly on the screen for an indefinite period for detailed explanation;

(2) contrasts and comparisons can be made most easily and, if necessary, remade to fit the mental response of the audience; (3) in subsequent discussion any one scene can be selected quickly and returned to the screen; (4) the still transparencies can be placed in an illuminator and the entire sequence can be studied at one time. Of course, the ideal is a motion picture and a series of still transparencies of the same operation. This is often impractical, however, due to the cost or the impossibility of repeating the exact operation.

To accomplish the most with slides, we find that a 300 or higher wattage projector with forced draft or other means of thorough ventilation is necessary. This type of equipment enables a lecturer to retain a slide in the projector indefinitely without damage and still secure good projection with sufficient light in the room for note taking.

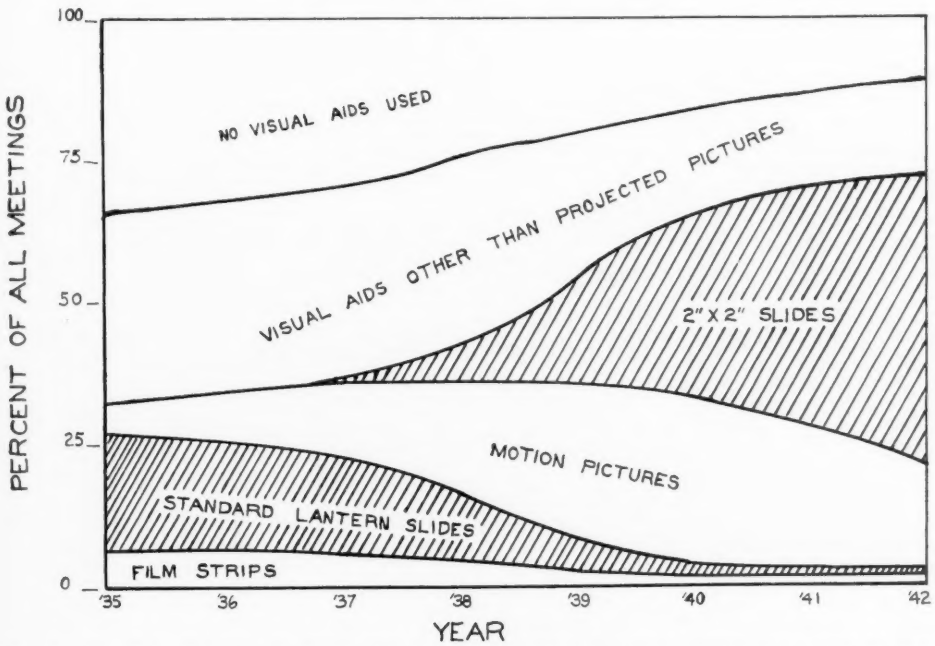


Fig. 3.—Trend in the use of visual aids in indoor subject matter meetings conducted by the Pennsylvania Agricultural Extension Service, 1935 to 1942.

One important factor in our plan is that assistance and encouragement are given for workers to produce their own visual aids. The development of the miniature camera, color film, and the exposure meter has been a great stimulus in this direction. The miniature camera, as we use it, is a lantern slide camera because color film is used, and when the shutter is clicked, a lantern slide is produced. This so simplifies the procedure of making lantern slides that over 80 of our workers are successfully producing these slides at the rate of 5,000 a year.

MINIATURE REFLEX CAMERA FOR PHOTOMICROGRAPHY

We find the 35 mm. film size reflex camera extremely practical for photomicrography. In this type of photography we employ a carbon arc light for illumination with either transmitted or reflected light, depending upon the

subject. The camera setup is as follows: The lens of the camera is replaced with a hinged microscope adapter which slips over the top of the microscope. The hinge enables the technician to swing the camera aside while searching for the proper field to be photographed. When this is determined, the camera is returned to the top of the microscope and focusing is completed through the

Fig. 4.

Fig. 5.

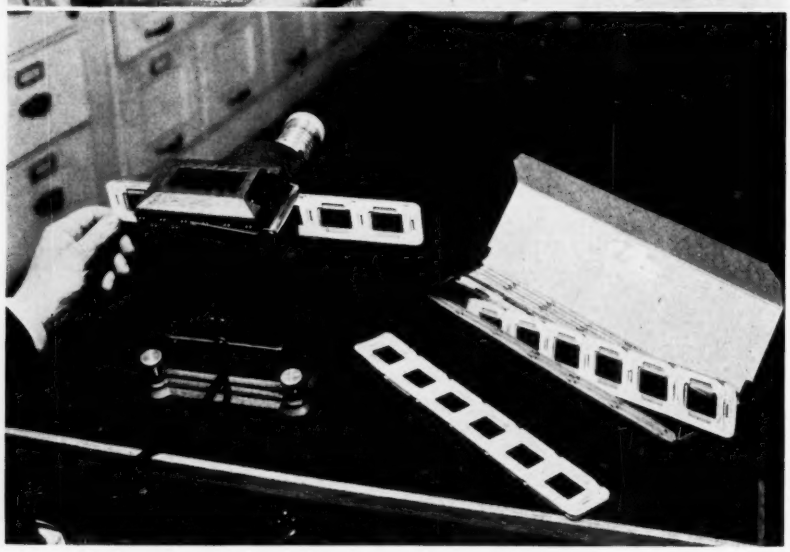


Fig. 4.—Filing 2 by 2 inch slides in 100 capacity boxes. Squares of cardboard with labels at the top are used for dividers.

Fig. 5.—The projector strip method of filing 2 by 2 inch slides.

reflex viewer. By using type A Kodachrome film and the carbon arc light, our experience indicates an exposure from one-tenth second to three seconds, based upon various light factors. We normally take three exposures, such as one-tenth, one-half, and one second, one-half, one, and two seconds, or one, two, and three seconds, depending upon our judgment of which light group fits the subject.

Fig. 6.

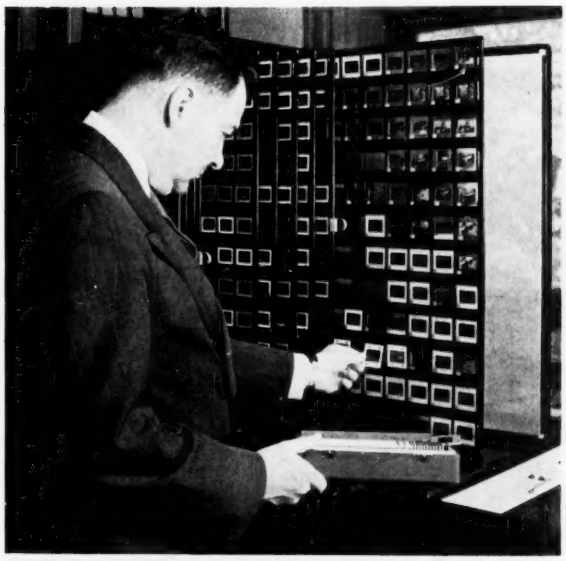


Fig. 7.

Fig. 6.—Sliding panels in a metal cabinet provide an efficient file for 2 by 2 inch slides.
Fig. 7.—Using the illuminator to arrange a series of slides for an illustrated talk.

The 2 by 2 inch Kodachrome transparencies can be duplicated successfully by the manufacturer at a cost of 25 cents each. The best duplicates are made from correctly or slightly underexposed views. Overexposures do not duplicate well. Color prints, measuring 2¼ by 3¼ inches, can be made from the transparencies by the manufacturer at a cost of 75 cents each. A 5 by 7½ inch

size is made for $3.50. Fairly close-up subjects with distinct lines photographed on 35 mm. Kodachrome can often be converted successfully to a 5 by 7 inch black-and-white negative for a monochrome contact print of publication quality.

EFFICIENT FILES A NECESSITY

Since many of our departments and county offices have from 200 to 800 of the 2 by 2 inch slides, efficient filing has become essential to the best utilization of these vast visual aid resources. We have several types of files in use: (1) the box type with partitions for individual slides; (2) the panel type in which slides are filed in panels or cardboard holders and viewed by holding them before a lighted background; (3) the projector strip file in which slides are held in a cardboard projector strip that is used as a slide carrier in the projector (see Figs. 4 to 6).

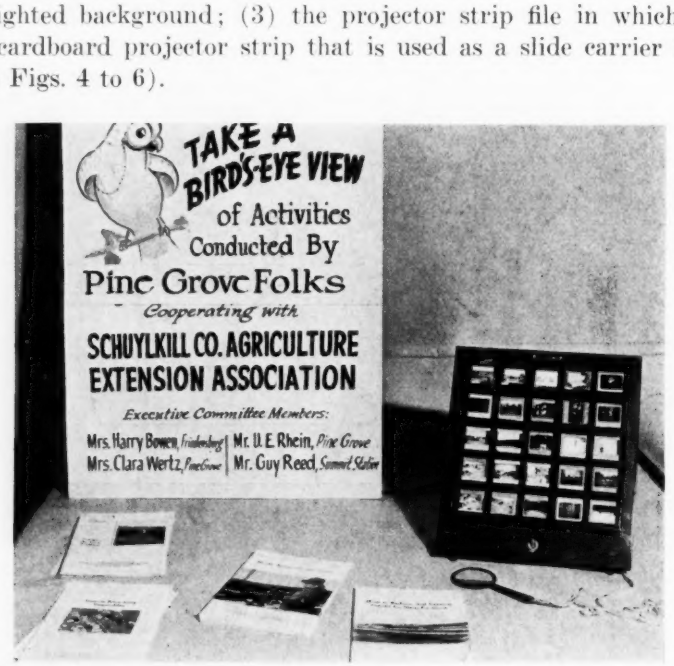


Fig. 8.—Inexpensive, simple and yet an extremely effective local community exhibit based upon the use of 2 by 2 inch slides in an illuminator. Note the magnifying glass on a string for use in looking at slides.

In general, a satisfactory file has the following characteristics: It is convenient to get slides into and out of the file with a minimum amount of time; it is not so complicated that great effort is required in keeping the file up to date; it is flexible in compartment spacing and new slides can be added without the continual handling of old slides. The object of an efficient slide filing system is to reduce slide handling and effort in filing to a minimum and at the same time make it possible to organize an illustrated talk quickly with all the material at hand. We have found that our workers who make the most of color slides in teaching are likely to accumulate at least 300 slides. To be most useful these must be in an efficient file. The cost of equipment for filing varies from 2 to 7 cents for each glass-covered slide, depending upon the system used.

THE ILLUMINATOR IN USE

In addition to a well-kept file of slides we find a relatively new piece of visual aid equipment called the *illuminator* useful in organizing a series of

these slides for lecture purposes. In simple terms, the illuminator is a ventilated boxlike structure with a white interior containing a 60 watt ordinary light bulb. The surface to be used for illuminating slides is of opal or ground glass. The slides are assembled on this surface.

With a 25 capacity slide illuminator available it is relatively easy to organize a series of slides for a lecture (Fig. 7). This piece of equipment eliminates the need of a preliminary showing of the slides on the screen, and it has the advantage of all slides being in view at one time. A magnifying glass can be used to enlarge the picture, if desired. Furthermore, we find the illuminator filled with color slides useful as the central feature of simple, inexpensive exhibits as shown in Fig. 8. In this we recognize the natural color slide as something more than a lantern slide to project onto a screen.

In our work we find a number of advantages in illuminated slides over projected pictures. The pictures can be viewed clearly in average indoor light without darkening the room. The need of carrying projection equipment and the difficulty of setup in small quarters filled with people are eliminated. Besides, a series of slides can be viewed and studied individually or as a sequence with all slides continually in view. On the other hand, the use of the illuminator is limited to situations where only a few persons look at the slides at one time, and it is impossible to show all types of views with equal effectiveness.

THE FUTURE

The 2 by 2 inch slide is definitely established as a visual aid medium of vast possibilities. As professional workers become more familiar with its many advantages the scope of its usefulness should continue to increase. It is likely to establish itself as an important supplement to the motion picture. For persons and institutions with limited budgets it will of necessity become the visual aid medium of primary consideration.

REFERENCES

1. Jeter, Hugh, and Hull, Wayne M.: Color Photography and Its Application in Medical Teaching, J. Lab. & Clin. Med. **23**: 753, 1937-1938.
2. Tomlinson, Wray J.: Clinical Laboratory Photographs With a New Method of Preparing Photomicrographs, J. Lab. & Clin. Med. **26**: 1064, 1941.
3. DeLucchi, Milton R.: 1941 Survey of Biological Photography, J. Biol. Photo. A. **10**: 137, 1942.
4. Brubaker, J. D., and Holinger, Paul H.: The Larynx, Bronchi and Esophagus in Kodachrome, J. Biol. Photo. A. **10**: 83, 1941.
5. Waller, R. K., and Breedis, C.: Photomicrography in Color, J. Biol. Photo. A. **10**: 71, 1941.
6. Footer, Wilson: 35 mm. X-ray Slides, J. Biol. Photo. A. **10**: 13, 1941.
7. Koch, Ferdinand L. P.: Color Photography of the Human Ocular Fundus, J. Biol. Photo. A. **9**: 119, 1941.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCULOSIS, Pulmonary, The Weltmann Reaction, Owen, W. F., Jr., and Van Ordstrand, H. S. Cleveland Clin. Quart. 9: 135, 1942.

The Weltmann coagulation band was determined in 85 normal persons and a remarkably constant result was obtained, i.e., 6 to 6½.

In 75 cases of pulmonary tuberculosis, followed for a period of three to four months, an attempt was made to define how closely the coagulation band reflected the clinical picture and whether there was any correlation between the band and the sedimentation rate. In this group of 75 cases, 25 were chronic, arrested inactive cases, and in these the Weltmann reaction consistently showed a "shift to the right" and was a better indication of the clinical status than the sedimentation rate. In the remaining 50 cases the Weltmann coagulation band followed the clinical picture in 32 cases, or only 64 per cent, while the sedimentation rate was a good index in 40 cases, or 79 per cent. When the cases were divided into far advanced and minimal, it was shown that in the 27 far-advanced cases the Weltmann reaction was reliable in only 50 per cent as compared with 84 per cent with the sedimentation test. In the moderately advanced group of 20 cases, the sedimentation rate showed 60 per cent reliability and the coagulation band 80 per cent. There were only three minimal cases studied; the sedimentation rate was of no value in any while the Weltmann test was reliable in all.

Neither the Weltmann reaction nor the sedimentation rate is completely reliable or infallible in the diagnosis and prognosis of pulmonary tuberculosis. In these cases the sedimentation rate has been a somewhat better index of the clinical status of the patient. In doubtful cases it may be of value to do both tests.

In the chronic, arrested, inactive cases the Weltmann reaction consistently showed a "shift to the right" and was a better indication of the clinical status than the sedimentation rate.

There is no definite correlation between the Weltmann coagulation band the sedimentation rate.

PNEUMONIA, Capsular Polysaccharide in the Blood of Patients With Pneumococcic, Bukantz, S. C., deGara, P. F., and Bullowa, J. G. M. Arch. Int. Med. 69: 191, 1942.

Systematic determination of capsular polysaccharide, agglutinin, and precipitin titers were made on samples of blood of 135 patients with pneumonia caused by pneumococci of types I (32 cases), II (4 cases), III (40 cases), IV (1 case), V (9 cases), VII (33 cases), and VIII (16 cases) in a series rotated for treatment with sulfapyridine, with serum or with the two in combination.

Capsular polysaccharide was detected in the blood of 16 patients, or 11.8 per cent of the entire series. Eleven of the 16 had type III, 3 had type VII, and one had type VIII pneumococcus pneumonia.

There was a significantly greater incidence of positive reactions for circulating capsular polysaccharide in the older age group, a tendency to greater incidence with increasing duration of disease, and an equal sex incidence.

The mortality for the patients in whose blood capsular polysaccharide was present was 62.5 per cent, while the mortality for all the patients with bacteriemia was only 29 per cent, and the mortality for the entire series was 11.8 per cent. The mortality for the patients without bacteriemia and without capsular polysaccharide in the blood was only 3 per cent.

Of the 10 patients whose serum gave a positive reaction for capsular polysaccharide and who were treated with sulfapyridine alone, 6 died. The illness of the 4 patients recovering

under sulfapyridine therapy alone ran a prolonged course, with a tendency to recurrence of fever, slow disappearance of capsular polysaccharide from the blood, and failure to produce antibody.

Of the 5 patients whose serum gave a positive reaction for capsular polysaccharide and who were treated with both sulfapyridine and serum, 3 died; two of these did not receive serum and sulfapyridine simultaneously, and the third received insufficient sulfapyridine. The 2 patients recovering after simultaneous serum and sulfapyridine therapy made much more satisfactory clinical responses. The sixteenth patient in whose blood capsular polysaccharide was detected died after treatment with serum alone.

CARTILAGE, A New Histochemical Reaction With High Specificity for Cartilage Cells, Hass, G. M. Arch. Path. 33: 174, 1942.

The fresh tissues, except when decalcification was necessary, was cut into sections with a freezing microtome set at 15 to 20 microns. The sections were divided into lots for preservation in water, alcohol, and solution of formaldehyde U.S.P. The sections in distilled water were subdivided into three groups. One group was used for immediate study. Sections of the second group were mounted on glass slides and dried rapidly in a vacuum desiccator. The third group of sections was placed in the refrigerator at 5° C.

It was impossible to obtain suitable sections of fresh bone, although osseous tissue often remained so attached to epiphyseal cartilages at the primary zones of ossification that a few observations could be made. Decalcification of formaldehyde-fixed or fresh tissue with 0.5 molar hydrochloric acid was necessary before good sections of bone were obtained.

Sections of the designated organs and tissues were exposed to the action of numerous combinations and concentrations of 0.5 molar hydrochloric acid, 0.5 molar sodium hydroxide, 0.20 molar sodium nitrite and 0.04 molar crystal violet. Optimum conditions for obtaining the desired reaction were as follows: A solution containing 10 c.c. of 0.5 molar hydrochloric acid, 4 c.c. of 0.04 molar crystal violet, and 1 c.c. of 0.20 molar sodium nitrite, or multiples thereof, was prepared. The reaction was allowed to proceed for forty-five minutes at room temperature. Then 0.5 molar sodium hydroxide was added slowly until a delicate fluorescence developed. Seven to 8 c.c. were required, the quantity being so adjusted that a fine precipitate formed in the reaction mixture about ten minutes after appearance of the fluorescence. After addition of the alkali, 20 c.c. of the reaction mixture was transferred to each of a desired number of 25 c.c. beakers. One section of tissue was placed in each beaker and kept there throughout the fifteen to twenty minutes required for the formation of a precipitate. After precipitation was complete, ether was poured on the surface of the solution. The sections were withdrawn from the reaction mixture through the ether layer, washed in distilled water, floated onto glass slides, blotted with filter paper, stained with aqueous hematoxylin, and mounted in glycerogelatin under a cover slip.

Similar results were obtained when the sections had been affixed to glass slides by preparatory desiccation in vacuo. When available in this form, the tissues were more easily handled than when they were floating in the solution.

This standard method was varied so that some data might be gathered with respect to the action of alcohol, acetone, ether, and 0.5 molar hydrochloric acid on the capacity of the tissue to react and on the stability of the reaction product once it had formed. By use of these reagents it was possible to make a microscopic study of cells prior to running the reaction, after a positive reaction had been obtained, and after eliminating all traces of the positive reaction. In other attempts to determine the nature of the reactive cytoplasmic component, the carmine stains for glycogen, the perosmic acid stain for lipids, the sudan IV stain for neutral glycerides, and the hematoxylin-eosin stains were prepared.

A positive reaction consisted of an aggregate of particles in the cytoplasmic area of a cell. The particles, which varied from 1 to 3 microns in diameter, were yellow, refractile, and discoid or spherical. They often had a biconcave form and always possessed curved margins.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Gould's Medical Dictionary*

IF THERE is one book that every medical library and every physician *must* have, it is a medical dictionary. This new edition of Gould, long a favorite, will be found abreast of all the many and varied advances which have brought new and varied terms to medical literature. Numerous tables and new illustrations, many in color, make this new edition highly acceptable. It is highly recommended.

Textbook of Bacteriology†

THROUGH twelve editions Jordan's *Bacteriology* has established its own place. Dr. Burrows has taken the place of the late Dr. Jordan in editing the thirteenth edition. It has been somewhat enlarged and, with minor exceptions, completely rewritten. Chapters devoted to highly specialized fields, such as soil, industrial and dairy bacteriology, have been regrouped in a chapter on Bacterial Physiology. The relation of bacteria to disease is considered in its broad aspect. This applies especially in the section on virulence and resistance.

The editor has been conservative in his treatment of nomenclature and classification. He uses no single taxonomic scheme, although he favors most of the terms adopted in Bergey's *Manual*.

The new edition gives every promise of enjoying the popularity of its predecessors.

Foreign Bodies Left in the Abdomen‡

THIS is a summary review of the entire literature of the subject, analyzed and discussed with regard to methods of prevention and to legal responsibilities.

The first record of a sponge left in the abdomen was in 1895, when a sea sponge was discovered. Sea sponges evidently were used quite frequently in the early days. Very few appear in the literature after 1900, although one was found in 1916, which apparently had been introduced in the same year.

In 1901 a towel, measuring one foot by two feet, was left in an abdomen.

The interval between loss and recovery of sponges is sometimes very long. After the fifth year secondary operations caused by sponges are usually due to intestinal complications, although nearly half of them become encapsulated, cause little or no trouble, and are found only incidentally in other operations.

Methods of preventing loss are discussed in much detail. These include sponge counts, metal tracers, and the continuous sponge.

Most of the instruments left in the abdomen are forceps, hemostats, and needles, although one report from Germany described a pair of spectacles.

Drains are lost often enough to be important. Some foreign bodies in the abdominal cavity were originally swallowed.

The legal decisions reviewed in considerable detail will be of greatest interest to any who have been unfortunate enough to have left articles in the abdomen.

---

*Medical Dictionary. By George M. Gould, A.M., M.D. Revised by C. V. Brownlow. Flexible, ed. 5, 1,528 pages, illustrated. Plain, $7.00; thumb index, $7.50. The Blakiston Co., Philadelphia.

†Textbook of Bacteriology. By Edwin O. Jordan, Ph.D., late Andrew McLeish Distinguished Service Professor of Bacteriology, University of Chicago; and William Burrows, Ph.D., Assistant Professor of Bacteriology, University of Chicago. Cloth, ed. 13, revised, 731 pages. W. B. Saunders Company, Philadelphia and London, 1941.

‡Foreign Bodies Left in the Abdomen. The Surgical Problems—Cases, Treatment Prevention. The Legal Problems—Cases, Decisions, Responsibilities. By Harry Sturgeon Crossen, M.D., School of Medicine, Washington University; and David Frederic Crossen, LL.B., School of Law, Washington University, St. Louis, Mo. Cloth, 762 pages, with 212 illustrations including 4 color plates. The C. V. Mosby Company, St. Louis, 1940.

Approved Laboratory Technic*

THE third edition of the *Approved Laboratory Technic* includes such new procedures as the quantitative determination of prothrombin, methods for semen examination, the determination of urobilinogen in the urine and feces, hippuric acid in the urine, serum lipase determination, the simplified Kolmer complement fixation test, the Eagle modification of the Wassermann, determination of vitamin C in urine and plasma, determination of sulfonamide compounds in the blood and urine, and determination of thiocyanates in the blood.

This book was originally prepared under the auspices of the American Society of Clinical Pathologists.

In addition to the authors, twenty-eight men have collaborated in the preparation of certain sections.

Science and Seizures†

A VERY readable popular book for the use of the patient with epilepsy or migraine, this work was written from the point of view of neuropsychiatry. The patient with epilepsy (Lennox prefers to call it seizures) will get a clearer understanding of his condition from perusal of this book and will be relieved of the feeling that he is stigmatized by his disease. For this purpose the book should be widely recommended.

Physicians, even neuropsychiatrists, will also enjoy it and, unless they have kept up with the most recent advances in the study of seizures, they will gain much from its perusal.

Dr. Lennox devotes considerable space to discussion of the importance and significance of electroencephalograms. Some workers in the field may feel that the generalizations concerning the significance of the electroencephalogram are somewhat premature, not as yet generally accepted. The deductions, however, are based upon fact as it is known today and certainly add to the interest of the volume.

Cardiac Classics‡

CARDIAC CLASSICS contains reprints and translations of fifty-two contributions by fifty-one men who have done most to advance our knowledge of cardiology. Aside from the fun that one derives from reading those essays which have made history in medicine, there is a utilitarian need for this type of volume for those who are writing today. Often quotations from earlier investigators are distorted successively by men who fail to go back to the original source for their quotations. The truth of this is so obvious to those who do much writing that examples need not be given. Often the misquotations actually change the entire sense of a remark.

Another realization came to the reviewer as he went through the pages of this excellent work: how few essays written by medical men deserve reprinting and rereading. How much print paper is wasted with rehash and unimportant stuff.

---

*Approved Laboratory Technic—Clinical Pathological, Bacteriological, Mycological, Parasitological, Serological, Biochemical and Histological. By John A. Kolmer, M.S., M.D., Dr. P.H., Sc.D., LL.D., L.H.D., F.A.C.P., Professor of Medicine, Temple University; Director of the Research Institute of Cutaneous Medicine, Philadelphia; formerly Professor of Pathology and Bacteriology, Graduate School of Medicine, University of Pennsylvania; and Fred Boerner, V.M.D., Assistant Professor of Bacteriology, School of Medicine and Graduate School of Medicine, University of Pennsylvania; Bacteriologist, Graduate Hospital, Philadelphia. Cloth, ed. 3, 921 pages, $8.00. D. Appleton-Century Company, New York and London, 1941.

†Science and Seizures: New Light on Epilepsy and Migraine. By William Gordon Lennox, M.D., Sc.D., Hon. Assistant Professor of Neurology, Harvard University Medical School; Visiting Neurologist, Boston City Hospital; President International League against Epilepsy; Vice President Laymen's League against Epilepsy; Secretary Harvard Epilepsy Commission. Cloth, 258 pages, with 10 illustrations, $2.00. Harper & Brothers, New York and London, 1941.

‡Cardiac Classics—A Collection of Classic Works on the Heart and Circulation With Comprehensive Biographic Accounts of the Authors. Fifty-Two Contributions by Fifty-One authors. By Fredrick A. Willius, M.D., M.S. in Med., Chief, Section of Cardiology, the Mayo Clinic; Professor of Medicine, the Mayo Foundation for Medical Education and Research, the Graduate School, the University of Minnesota; and Thomas E. Keys, A.B., M.A., Reference Librarian, the Mayo Clinic; formerly Carnegie Fellow, the Graduate Library School, the University of Chicago. Cloth, 858 pages, $10.00. The C. V. Mosby Company, St. Louis, 1941.

The work starts with William Harvey and ends with Herrick's description of coronary occlusion.

One is disappointed in not seeing an essay from the pen of Thomas Lewis, but it is interesting to observe that there are more index references to his name than to that of any other cardiologist outside the favored fifty-one.

Dynamics of Inflammation*

AN OUTSTANDING contribution to our knowledge of inflammation, this book differs from the older pathologic concept in the sense that it is dynamic rather than static. The author analyzes the physiology and pathological physiology of the migration of leucocytes from the capillaries and their activities in areas of infection and damage, and associated chemical changes as well as modifications in these activities brought about by immunity and allergy. This has been the field of Menkin's original investigations for many years. In addition he brings to the monograph a wealth of his own material.

Although the volume represents a tremendous amount of original investigations, Dr. Menkin's conclusions, his interpretations of the mechanism of inflammation, are such as are now widely accepted. The evidence presented in favor of acceptance of the new trophic substance which he has named leukotaxine is logical.

ITEM

Antipneumococcic Action of Cinchona Derivatives

Mellon Institute, Pittsburgh, Pa., is distributing gratis to all interested specialists who request them copies of a publication entitled "Structure and Antipneumococcic Activity in the Cinchona Series."

Erratum

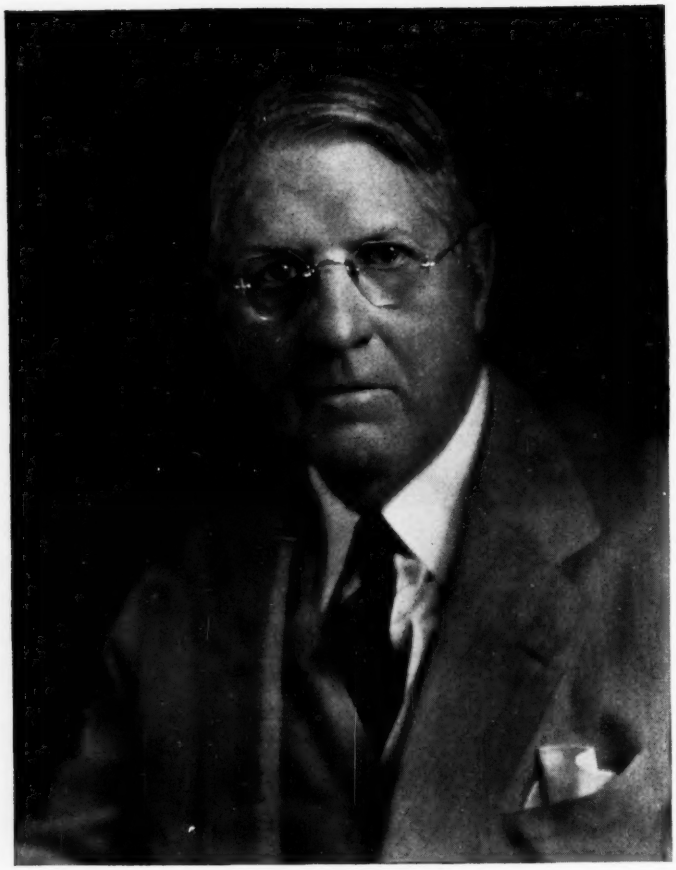
The first paragraph on page 1458 of the August issue of the JOURNAL, in an article entitled "Studies on the Clinical Significance of the Serum Proteins" by Benjamin M. Kagan, M.D., Richmond, Va., should read:

"There are in existence several colorimetric methods which, while less difficult to use, do not approach the simplicity of usual routine tests, *and* some of which are subject to errors up to 10 per cent."

SIMPSON MEMORIAL INSTITUTE
ANN ARBOR, MICH.

---

*Dynamics of Inflammation. An Inquiry Into the Mechanism of Infectious Processes. By Valy Menkin, Department of Pathology, Harvard University Medical School. Cloth, 244 pages. $4.50. The Macmillan Company, New York, 1940.



Charles Virgil Mosby
1876-1942

Founder of The Journal of Laboratory and Clinical Medicine

The Editors of

The Journal of Laboratory and Clinical Medicine

mourn the passing of its founder

Charles Virgil Mosby

who has contributed much to the progress of medicine in the building of a publishing medium second to none in its special field, thus providing facilities for interchange of ideas and perpetuation of accomplishments in the sciences

"Medicine and surgery would still be primitive crafts had it not been for a few dauntless souls who were willing to record faithfully, by the written word, the results of their findings."

"The sculptor needs the granite block out of which to carve the image that has taken form and substance in his brain. On its unyielding face he carves the creatures of his imagination. We are the sculptors that carve day by day our form and likeness upon the rocks of time. Overcome the difficulties, surmount the obstacles, break down the barriers, and you carve deep and lasting. You cut your image so deeply upon the hearts and into the souls of men that time itself will never efface your memory."

—*From the writings of C. V. Mosby*